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(57) Abstract

The present invention relates to immunogenic Toxoplasma gondii proteins, to T. gondii nucleic acid molecules, including those that encode such proteins and to antibodies raised against such proteins. The present invention also includes methods to obtain such proteins, nucleic acid molecules and antibodies. Also included in the present invention are compositions comprising such proteins, nucleic acid molecules and/or antibodies, as well as the use of such compositions to inhibit oocyst shedding by cats due to infection with T. gondii. The present invention also includes the use of certain T. gondii—based antisera to identify such nucleic acid molecules and proteins, as well as nucleic acid molecules and proteins identified by such methods. The present invention also relates to methods for the detection of cysts and oocysts.

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TOXOPLASMA GONDII PROTEINS, CORRESPONDING NUCLEIC ACID MOLECULES, AND USES THEREOF

FIELD OF THE INVENTION

The present invention relates to *Toxoplasma gondii* nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins and methods to identify such nucleic acid molecules, proteins or antibodies. The present invention also includes compositions comprising such nucleic acid molecules, proteins and antibodies, as well as their use for inhibiting oocyst shedding by cats infected with *T. gondii* and for protecting animals from diseases caused by *T. gondii*.

BACKGROUND OF THE INVENTION

Various attempts to develop a vaccine to both the asexual systemic stage and the sexual entero-epithelial stage of the Toxoplasma life cycle have been reported over the last thirty years (Hermentin, K. and Aspock, H. (1988), Zbl. Bakt. Hyg. A, 269:423-436). These attempts can be grouped into the following categories: 1) immunization with 15 whole killed organism, 2) immunization with selected antigens, either purified native or recombinant protein, 3) immunization with attenuated strains, and 4) immunization with irradiated organisms. Little success has been achieved with immunizations using whole killed organism (Frenkel, J.K. and Smith, D.D. (1982), Journal of Parasitology, 68:744-748). Partial success has been observed with the pure native protein P30 (Bulow, R., and Boothroyd, J. C. (1991), J. Immunol. 147:3496) and with selected fractions of 20 parasite lysates (Lunden, A. Lovgren, K. Uggla, A., and Araujo, F.G.; (1993) Infection and Immunity, 61: 2639-2643). However, attempts with purified recombinant antigens have not been successful (Lunden, A., Parmley, S.F., Bengtsson, K.L. and Araujo, F.G. (1997) Parasitology Research, 83:6-9). Studies with irradiated organisms have reported 0-90% protection and are complicated by the uncertainty of truly inactivated irradiated 25 preparations. Effective vaccines have been produced using attenuated strains. Two such mutant strains, ts-4 (Waldeland, H., Pfefferkorn, E.R., and Frenkel, J.K. (1983), Journal of Parasitology, 69:171-175) and S48 (Hartley, W.J. and Marshall, S.C. (1957), New Zealand Veterinary Journal, 5:119-124), successfully protect animals against the 30 asexual systemic disease. These strains are delivered in the tachyzoite form and do not

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protect cats from oocyst shedding. Another strain, T-263 (Frenkel, J.K.; Pfefferkorn, E.R.; Smith, D.D.; and Fishback, J.L. (1991), *American Journal of Veterinary Research*, 52:759-763) is an oocyst minus strain, but was shown to progress through most of the entero-epithelial stages in the cat intestine. Exposure to this strain induces immunity in the cat to oocyst shedding upon subsequent challenge. There remains a need for an effective vaccine for prevention of the diseases caused by infection with *Toxoplasma gondii*.

SUMMARY OF THE INVENTION

The present invention relates to novel compositions and methods to inhibit *Toxoplasma gondii* (*T. gondii*) oocyst shedding by cats, thereby preventing the spread of *T. gondii* infection. According to the present invention there are provided isolated immunogenic *T. gondii* proteins and mimetopes thereof; *T. gondii* nucleic acid molecules, including those that encode such proteins; recombinant molecules including such nucleic acid molecules; recombinant viruses including such nucleic acid molecules; recombinant cells including such nucleic acid molecules; and antibodies that selectively bind to such immunogenic *T. gondii* proteins.

The present invention also includes methods to obtain and/or identify proteins, nucleic acid molecules, recombinant molecules, recombinant viruses, recombinant cells, and antibodies of the present invention. Also included are compositions comprising such proteins, nucleic acid molecules, recombinant molecules, recombinant viruses, recombinant cells, and antibodies, as well as use of such compositions to inhibit *T. gondii* oocyst shedding by cats infected with *T. gondii*, or for preventing *T. gondii* infection in an animal.

The present invention further includes the use of the nucleic acid molecules or proteins of the present invention as diagnostic reagents for the detection of *T. gondii* infection. In a preferred embodiment, the present invention includes a novel detection method and kit for detecting *T. gondii* oocysts in the feces of *T. gondii* infected cats.

One embodiment of the present invention is an isolated nucleic acid molecule encoding an immunogenic *T. gondii* protein that can be identified by a method that includes the steps of: a) immunoscreening a *T. gondii* genomic expression library or

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cDNA expression library with an antiserum, including an antiserum derived from intestinal secretions; and b) identifying a nucleic acid molecule in the library that expresses a protein that selectively binds to an antibody in the antiserum. Antisera to be used for screening include antiserum raised against *T. gondii* oocysts, antiserum raised against *T. gondii* bradyzoites, antiserum raised against *T. gondii* infected cat gut, and antiserum isolated from a cat immune to *T. gondii* infection. Another embodiment is an isolated immunogenic *T. gondii* protein that can be identified by a method that includes the steps of: a) immunoscreening a *T. gondii* genomic expression library or cDNA expression library with such an antiserum; and b) identifying a protein expressed by the library that selectively binds to antibodies in the antiserum. Also included are methods to identify and isolate such nucleic acid molecules and proteins.

The present application also includes an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene that includes a nucleic acid sequence cited in Table 1. Also included in the present invention is an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene that includes a nucleic acid molecule cited in Table 1. Preferred nucleic acid molecules encode immunogenic *T. gondii* proteins. More preferred nucleic acid molecules are those cited in Table 1.

The present invention also relates to recombinant molecules, recombinant viruses and recombinant cells that include an isolated nucleic acid molecule of the present invention. Also included are methods to produce such nucleic acid molecules, recombinant molecules, recombinant viruses and recombinant cells.

Another embodiment of the present invention is an isolated immunogenic protein encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene (i.e., with either the coding strand or the non-coding strand) comprising a nucleic acid sequence cited in Table 1 and/or a nucleic acid molecule cited in Table 1. Note that the nucleic acid molecule hybridizes with the non-coding strand of the gene, that is, with the complement of the coding strand of the gene. A preferred protein is an immunogenic *T. gondii* protein. More preferred proteins are those encoded

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by nucleic acid molecules cited in Table 1. Also preferred are the proteins cited in Table 1.

The present invention also relates to: mimetopes of immunogenic *T. gondii* proteins and isolated antibodies that selectively bind to immunogenic *T. gondii* proteins or mimetopes thereof. Also included are methods, including recombinant methods, to produce proteins, mimetopes and antibodies of the present invention.

Yet another embodiment of the present invention is a composition to inhibit T. gondii oocyst shedding in a cat due to infection with T. gondii. Such a composition includes one or more of the following protective compounds: an isolated immunogenic T. gondii protein encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene comprising a nucleic acid sequence cited in Table 1, and specifically with the non-coding-strand of that gene; an isolated antibody that selectively binds to said immunogenic T. gondii protein; and an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene comprising a nucleic acid sequence cited in Table 1. Such a composition can also include an excipient, adjuvant or carrier. Preferred compositions comprising a nucleic acid molecule of the present invention include genetic vaccines, recombinant virus vaccines and recombinant cell vaccines. Also included in the present invention is a method to protect an animal, including a human, from disease caused by T. gondii, comprising the step of administering to the animal a composition of the present invention. Preferred animals to treat are cats in order to prevent oocyst shedding caused by T. gondii infection.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides for isolated immunogenic *T. gondii* proteins, isolated *T. gondii* nucleic acid molecules including those encoding such *T. gondii* proteins, recombinant molecules comprising such nucleic acid molecules, recombinant viruses comprising such nucleic acid molecules, cells transformed with such nucleic acid molecules (i.e., recombinant cells), and antibodies that selectively bind to immunogenic *T. gondii* proteins. As used herein, the terms isolated immunogenic *T. gondii* protein and isolated nucleic acid molecule refer to an immunogenic *T. gondii* protein and a *T. gondii* nucleic acid molecule, respectively, derived from *T. gondii* which can be obtained from its natural source or can be produced using, for example, recombinant nucleic acid

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technology or chemical synthesis. Also included in the present invention is the use of these proteins, nucleic acid molecules, and antibodies as compositions to protect animals from diseases caused by *T. gondii* and to inhibit *T. gondii* oocyst shedding in cats. As used herein, a cat refers to any member of the cat family (i.e., Felidae), including domestic cats, wild cats and zoo cats. Examples of cats include, but are not limited to, domestic cats, lions, tigers, leopards, panthers, cougars, bobcats, lynx, jaguars, cheetahs, and servals. A preferred cat to protect is a domestic cat. Further included in the present invention is the use of these proteins, nucleic acid molecules and antibodies for the detection of *T. gondii* infection in an animal or as targets for the development of chemotherapeutic agents against parasitic infection.

Immunogenic *T. gondii* protein and nucleic acid molecules of the present invention have utility because they represent novel targets for anti-parasite vaccines or chemotherapeutic agents. Compositions of the present invention can also be used as reagents for the diagnosis of *T. gondii* infection in cats and other animals, including humans. The products and processes of the present invention are advantageous because they enable the inhibition of *T. gondii* oocyst shedding in cats, the definitive hosts for *T. gondii* (i.e., the animals in which *T. gondii* reproduction takes place). It is to be noted that the proteins and nucleic acid molecules of the present invention have uses beyond eliciting an immune response despite denoting proteins of the present invention as immunogenic proteins.

As described in more detail in the Examples, it was very difficult to isolate a nucleic acid molecule encoding an immunogenic T. gondii protein selectively bound by antisera directed against T. gondii intestinal stages. Such stages are preferred because they represent the sexual cycle of T. gondii, the preferred target for development of a composition to inhibit oocyst shedding. Unfortunately, however, the T. gondii sexual cycle cannot currently be reproduced in culture, and, there is not a simple method by which to produce a cDNA (i.e., complementary DNA) library containing only T. gondii nucleic acid molecules of various stages of the sexual cycle. For example, the infected cat gut is the source of many of the sexual stages of T. gondii, and, as such, material to be used in identifying T. gondii immunogenic proteins are contaminated with cat

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material. The present invention describes the development of new techniques to isolate and identify nucleic acid molecules encoding immunogenic *T. gondii* proteins. These techniques include (a) the isolation and enrichment of antisera against a variety of *T. gondii* life stages, several of which are only present in infected cats, at least predominantly in infected cat guts, and (b) the use of such antisera to screen cDNA and genomic expression libraries to identify nucleic acid molecules that express *T. gondii* proteins that selectively bind to such antisera.

One embodiment of the present invention is an isolated protein that includes an immunogenic *T. gondii* protein. It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, a protein refers to one or more proteins or at least one protein. The terms "a" (or "an"), "one or more" and "at least one" can be used interchangeably herein. It is also to be noted that the terms "comprising", "including", and "having" can be used interchangeably. According to the present invention, an isolated, or biologically pure, protein is a protein that has been removed from its natural milieu. The terms "isolated" and "biologically pure" do not necessarily reflect the extent to which the protein has been purified. An isolated protein of the present invention can be obtained from its natural source, can be produced using recombinant DNA technology, or can be produced by chemical synthesis.

An isolated protein of the present invention, including a homolog, can be identified in a straight-forward manner by the protein's ability to elicit an immune response against a naturally occurring *T. gondii* protein. Examples of *T. gondii* immunogenic proteins include proteins in which amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide), inserted, inverted, substituted and/or derivatized (e.g., by glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitoylation, amidation and/or addition of glycerophosphatidyl inositol) such that the homolog includes at least one epitope capable of eliciting an immune response against a *T. gondii* immunogenic protein, and/or of binding to an antibody directed against a *T. gondii* immunogenic protein. That is, when the homolog is administered to an animal as an immunogen, using techniques known to those skilled in the art, the animal will produce an immune response against at least one epitope of a *T.*

be measured using techniques known to those skilled in the art. As used herein, the term "epitope" reters to the smallest portion of a protein or other antigen capable of selectively binding to the antigen binding site of an antibody or a T-cell receptor. It is well accepted by those skilled in the art that the minimal size of a protein epitope is about four to six amino acids. As is appreciated by those skilled in the art, an epitope can include amino acids that naturally are contiguous to each other as well as amino acids that, due to the tertiary structure of the natural protein, are in sufficiently close proximity to form an epitope. According to the present invention, an epitope includes a portion of a protein comprising at least about 4 amino acids, at least about 5 amino acids, at least about 20 amino acids, at least about 30 amino acids, at least about 35 amino acids, at least about 40 amino acids, at least about 50 amino acids, at least about 100 amino acids, at least about 50 amino acids, at least about 100 amino acids, at least about 300 amino acids, at least about 200 amino acids, at least about 200 amino acids, at least about 300 amino acids.

Immunogenic T. gondii protein homologs can be the result of natural allelic variation or natural mutation. Immunogenic T. gondii protein homologs of the present invention can also be produced using techniques known in the art including, but not limited to direct modifications to the protein or modifications to the gene encoding the protein using tor example, classic or recombinant DNA techniques to effect random or targeted matagenesis

Protein include a mucleic acid molecule encoding an immunogenic *T. gondii* protein include acid sequences related to a natural *T. gondii* gene. As used herein, a *T. sondii* gene includes all regions of the genome related to the gene, such as regulatory terrors that control production of the immunogenic *T. gondii* protein encoded by the gene (for example, transcription, translation or post-translation control regions) as well as the coding region itself, and any introns or non-translated coding regions. As used herein, a gene that "includes" or "comprises" a sequence may include that sequence in one contiguous array, or may include the sequence as fragmented exons. As used herein, the term "coding region" refers to a continuous linear array of nucleotides that

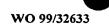
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translates into a protein. A full-length coding region is that coding region that is translated into a full-length protein, i.e., a complete protein as would be initially translated in its natural milieu, prior to any post-translational modifications.

In one embodiment, a *T. gondii* gene of the present invention includes at least one of the nucleic acid molecules cited in Table 1 (i.e., the cited nucleic acid molecules). The coding strands of the cited nucleic acid molecules are represented, respectively, by the nucleic acid sequences (i.e., the cited nucleic acid sequences) shown in Table 1. Also presented in Table 1 are the deduced amino acid sequences encoded by each of the cited nucleic acid molecules (i.e., the cited amino acid sequences) and the protein name designations (i.e., the cited proteins).

]	Nucleic Acid	Amino Acid	
SEQ ID NO	TYPE	Molecules	Molecules	Original Designation
	<u>:</u> T	: T		
1	DNA	nTG1 ₃₅₇		Tg-41
2	Protein		PTG1 ₁₁₉	PTG-41
3	DNA	nTG2 ₃₃₉		Tg-45
4	Protein		PTG2 ₁₀₈	PTG-45
5	DNA	nTG4 ₅₂₆		Tg-50
6	Protein		PTG4 ₁₇₅	PTG-50
7	cDNA	nTG4 ₁₄₇₈		Tg-50c
8	Protein		PTG4 ₃₈₁	PTG-50c
9	DNA	nTG5 ₆₅₇		Q2-4
10	Protein		PTG5 ₂₁₉	PQ2-4
11	cDNA	nTG5 ₁₀₂₉		Q2-4c
12	Protein		PTG5 ₂₇₃	PQ2-4c
13	DNA	nTG6 ₄₂₅		Q2-9
14	Protein		PTG6 ₁₄₂	PQ2-9
15	DNA	nTG7 ₄₁₇		Q2-10
16	Protein		PTG7 ₁₃₉	PQ2-10
17	DNA	nTG8 ₅₀₇		Q2-11
18	Protein		PTG8 ₅₁	PQ2-11
19	DNA	nTG9 ₇₁₈		4499-9
20	Protein		PTG9 ₉₉	P4499-9
21	DNA	nTG10 ₄₄₁		4604-2
22	Protein		PTG10 ₁₄₇	P4604-2
23	DNA	nTG11 ₄₂₈		4604-3
24	Protein		PTG11 ₁₃₄	P4604-3
25	DNA	nTG13 ₂₈₂		4604-5
26	DNA	nTG15 ₃₀₄		4604-10
27	Protein		PTG15 ₁₀₁	P4604-10
28	DNA	nTG16 ₂₈₄		4604-17
29	Protein		PTG16 ₉₅	P4604-17
30	DNA	nTG17 ₆₉₀		4604-54
31	Protein		PTG17 ₂₃₀	P4604-54
32	DNA	nTG18 ₃₁₃		4604-62
33	Protein		PTG18 ₅₄	P4604-62
34	DNA	nTG19 ₃₈₉		4604-63
35	Protein		PTG19 ₆₅	P4604-63

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BNSDOCID: <WO___9932633A1_I_>

Table 1

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SEQ ID NO	TYPE	Nucleic Acid Molecules	Amino Acid Molecules	Original Designation
	:		!	- Tongina Designation
36	DNA	nTG21 ₅₄₈		4604-69
37	Protein		PTG21 ₁₈₃	P4604-69
38	DNA	nTG22 ₃₁₀		BZ1-2
39	Protein		PTG22 ₉₅	PBZ1-2
40	DNA	nTG23 ₂₂₀		BZ1-3
41	Protein		PTG23 ₇₃	PBZ1-3
42	DNA	nTG24 ₆₄₂		BZ1-6
43	Protein		PTG24 ₃₄	PBZ1-6
44	DNA	nTG25 ₃₈₁		BZ2-3
45	Protein		PTG25 ₂₇	PBZ2-3
46	DNA	nTG26 ₄₃₂		BZ2-5
47	Protein		PTG26 ₈₅	PBZ2-5
48	DNA	nTG27 ₂₈₂		BZ3-2
49	Protein		PTG27 ₃₅	PBZ3-2
50	DNA	nTG28 ₄₆₆		BZ4-3
51	Protein		PTG28 ₇₁	PBZ4-3
52	DNA	nTG30 ₅₃₉		BZ4-6
53	Protein		PTG30 ₂₀	PBZ4-6
54	DNA	nTG31 ₁₂₃₃		AMX/I-5
55	DNA	nTG32 ₄₁₁		AMX/I-6
56	Protein		PTG32 ₆₀	PAMX/I-6
57	DNA	nTG33 ₄₄₁		AMX/I-7
58	Protein		PTG33 ₁₁₈	PAMX/I-7
59	DNA I	nTG34 ₄₉₁		AMX/I-9
60	Protein		PTG34 ₃₄	PAMX/I-9
61	DNA 1	nTG35 ₃₈₇		AMX/I-10
62	Protein		PTG35 ₁₂₉	PAMX/I-10
63	DNA	nTG36 ₄₁₇		AMI-23
64	Protein		PTG36 ₁₃₉	PAMI-23
65	DNA r	nTG37 ₄₁₆	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	AMI-24
66	Protein		PTG37 ₁₃₈	PAMI-24
67	DNA r	nTG38 ₅₀₀		AMI-28
68		TG40 ₃₂₁		AMI-47
69	Protein			PAMI-47
70	DNA r	TG41 _{513+C86}		OC-1
71	Protein			POC-1

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Table 1

SEQ ID NO	TYPE	Nucleic Acid Molecules	Amino Acid Molecules	Original Designation
72	DNA	nTG42 ₅₂₈		OC-2
73	Protein		PTG42 ₁₇₆	POC-2
74	DNA	nTG43 ₃₇₅		OC-13
75	Protein		PTG43 ₁₂₅	POC-13
76	DNA	nTG44 ₅₄₃		OC-14
77	Protein		PTG44 ₈₉	POC-14
78	DNA	nTG45 ₅₇₃		OC-22
79	Protein		PTG45 ₁₉₁	POC-22
80	DNA	nTG46 ₁₈₃₅		OC-23
81	Protein		PTG46 ₆₁₂	POC-23
82	DNA	nTG48 ₆₀₄		4CQA7f
83	Protein		PTG48 ₁₁₂	P4CQA7f
84	DNA	nTG48 ₅₄₉		4CQA7r
85	DNA	nTG49 ₂₇₀		4CQA11
86	Protein		PTG49 ₉₀	P4CQA11
87	DNA	nTG50 ₃₀₆		4CQA19
88	Protein		PTG50 ₁₀₂	P4CQA19
89	DNA	nTG51 ₈₀₄		4CQA21
90	Protein		PTG51 ₂₆₈	P4CQA21
91	DNA	nTG52 ₈₆₇		4CQA22
92	Protein		PTG52 ₂₈₉	P4CQA22
93	DNA	nTG53 ₁₄₃₄		4CQA24
94	Protein	ļ	PTG53 ₁₆₄	P4CQA24
95	DNA	nTG54 ₆₈₀		4CQA25
96	Protein		PTG54 ₂₂₇	P4CQA25
97	DNA	nTG55 ₂₉₆		4CQA26
98	Protein		PTG55 ₉₉	P4CQA26
99	DNA	nTG56 ₇₂₃		4CQA27
100	Protein		PTG56 ₅₃	P4CQA27
101	DNA	nTG57 ₂₇₀		4CQA29
102	Protein		PTG57 ₉₀	P4CQA29
103	DNA	nTG58 ₅₀₃		R8050-2
104	Protein		PTG58 ₆₂	PR8050-2
105	DNA	nTG60 ₃₂₂		R8050-5
106	Protein		PTG60 ₇₃	PR8050-5

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		Nucleic Acid	Amino Acid	
SEQ ID NO	TYPE	Molecules	Molecules	Original Designation
107	DNA	nTG61 ₃₉₀		R8050-6
108	Protein	111 00 1390	PTG61 ₆₇	PR8050-6
109	DNA	nTG62 ₆₉₉	1 700167	M2A1
110	Protein	Oozagg	PTG62 ₂₃₃	PM2A1
111	DNA	nTG63 ₄₁₉	1 1 2 2 2 3 3	M2A2
112	Protein	111 000419	PTG63 ₁₄₀	PM2A2
113	DNA	nTG64 ₃₀₃	1 1 0 0 0 1 4 0	M2A3
114	Protein	111 004303	PTG64 ₁₀₁	PM2A3
115	DNA	nTG65 ₆₉₆	1 1 3 3 7 101	M2A4
116	Protein	111 000696	PTG65 ₂₃₂	PM2A4
117	DNA	nTG66 ₁₇₃	1 1 3 3 2 3 2	M2A5
118	Protein	111 G00 ₁₇₃	PTG66 ₅₈	PM2A5
119	DNA	nTG67 ₃₆₉	F 1 G 0 0 58	M2A6
		11 GO7 369	PTG67 ₁₂₃	PM2A6
120	Protein	aTC69	1 1 307 123	M2A7
121	DNA	nTG68 ₅₆₆	PTG68 ₆₁	PM2A7
122	Protein	-TCC0	P1G00 ₆₁	M2A11
123	DNA	nTG69 ₆₁₆	DTCCC	
124	Protein		PTG69 ₂₀₅	PM2A11
125	DNA	nTG70 ₇₆₂	107070	M2A16
126	Protein		PTG70 ₂₅₄	PM2A16
127	DNA	nTG71 ₂₃₆		M2A18
128	Protein		PTG71 ₇₉	PM2A18
129	DNA	nTG72 ₅₆₉		M2A19
130	Protein		PTG72 ₁₉₀	PM2A19
131	DNA	nTG73 ₂₃₂		M2A20
132	DNA	nTG74 ₂₇₆		M2A21
133	Protein		PTG74 ₉₂	PM2A21
134	DNA	nTG75 ₃₀₉		M2A22
135	Protein		PTG75 ₁₀₃	PM2A22
136	DNA	nTG76 ₅₃₄		M2A23
137	Protein		PTG76 ₁₇₈	PM2A23
138	DNA	nTG76 ₄₂₃		M2A23
139	DNA	nTG77 ₃₂₇		M2A24
140	Protein		PTG77 ₁₀₉	PM2A24
141	DNA	nTG78 ₄₄₄		M2A25
142	Protein		PTG78 ₁₄₈	PM2A25

SEQ ID NO	TYPE	Nucleic Acid Molecules	Amino Acid Molecules	Original Designation
	<u> </u>			
143	DNA	nTG79 ₉₂₈		M2A29
144	Protein		PTG79 ₁₉	PM2A29
265	DNA	nTG22 _{310a}	 	BZ1-2-a
266	Protein		PTG22 _{95a}	PBZ1-2-a
267	DNA	nTG64 _{303a}		M2A3-a
268	Protein		PTG64 _{101a}	PM2A3-a
269	DNA	nTG71 _{236a}		M2A18-a
270	Protein		PTG71 _{79a}	PM2A18-a
271	DNA	nTG6 _{425a}		Q2-9-1-a
272	Protein		PTG6 _{142a}	PQ2-9-a
273	DNA	nTG41 _{513a}		OC-1-a
274	Protein		PTG41 _{171a}	POC-1-a
282	cDNA	nTG ₁₂₂₅		MGIS42
283	Protein		PTG ₂₈	PMGIS42
284	DNA	nTG ₁₂₂₅		rc
292	cDNA	nTG ₁₅₇₃		MGIS44
293	Protein		PTG ₇₃	PMGIS44
294.	DNA	nTG ₁₅₇₃		rc
306	cDNA	nTG ₂₄₁₇		MGIS48
307	Protein		PTG ₉	PMGIS48
308	DNA	nTG ₂₄₁₇		rc
311	cDNA	nTG ₁₇₈₅		MGIS65
312	Protein		PTG ₂₄	PMGIS65
313	DNA	nTG ₁₇₈₅		rc
338	DNA	nTG ₆₄₇		511-44 genomic
339	DNA	nTG ₆₄₇		rc
340	cDNA	nTG ₈₆₇		511-44 coding region
341	Protein		PTG ₂₈₈	P511-44
342	DNA	nTG ₈₆₇	- 200	rc
343	cDNA	nTG ₁₃₉₇		511-44cDNA
345	DNA	nTG ₁₃₉₇		rc

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It should be noted that because nucleic acid sequencing technology is not entirely error-free, the nucleic acid sequences disclosed in the present invention (as well as other nucleic acid and protein sequences presented herein) represent the apparent nucleic acid sequences of the nucleic acid molecules encoding *T. gondii* proteins of the present invention. The nucleic acid molecules cited in Table 1 also include the complementary (i.e., apparently non-coding) strands. As used herein the terms "complementary strand" and "complement" refer to the nucleic acid sequence of the DNA strand that is fully complementary to the DNA strand having the listed sequence, which can easily be determined by those skilled in the art. Likewise, a nucleic acid sequence complement of any nucleic acid sequence of the present invention refers to the nucleic acid sequence of the nucleic acid strand that is fully complementary to (i.e., can form a complete double helix with) the strand for which the sequence is cited. Production of the cited nucleic acid molecules is disclosed in the Examples as are methods to obtain nucleic acid sequences of the coding strands of such molecules and the amino acid sequences deduced therefrom.

In another embodiment, a T. gondii gene or nucleic acid molecule can be a naturally occurring allelic variant that includes a similar but not identical sequence to the cited nucleic acid molecules. A naturally occurring allelic variant of a T. gondii gene including any of the above-listed nucleic acid sequences is a gene that occurs at essentially the same locus (or loci) in the genome as the gene including at least one of the above-listed sequences, but which, due to natural variations caused by, for example, mutation or recombination, has a similar but not identical sequence. Because natural selection typically selects against alterations that affect function, allelic variants usually encode proteins having similar activity to that of the protein encoded by the gene to which they are being compared. Allelic variants of genes or nucleic acid molecules can also comprise alterations in the 5' or 3' untranslated regions of the gene (e.g., in regulatory control regions), or can involve alternative splicing of a nascent transcript, thereby bringing alternative exons into juxtaposition. Allelic variants are well known to those skilled in the art and would be expected to be found within a given T. gondii organism or population, because, for example, the genome goes through a diploid stage, and sexual reproduction results in the reassortment of alleles.

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In one embodiment of the present invention, an isolated immunogenic *T. gondii* protein is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions to a gene encoding an immunogenic *T. gondii* protein. The minimal size of a *T. gondii* protein of the present invention is a size sufficient to be encoded by a nucleic acid molecule capable of forming a stable hybrid (i.e., hybridizing under stringent hybridization conditions) with the complementary sequence of a nucleic acid molecule encoding the corresponding natural protein. The size of a nucleic acid molecule encoding such a protein is dependent on the nucleic acid composition and the percent homology between the *T. gondii* nucleic acid molecule and the complementary nucleic acid sequence. It can easily be understood that the extent of homology required to form a stable hybrid under stringent conditions can vary depending on whether the homologous sequences are interspersed throughout a given nucleic acid molecule or are clustered (i.e., localized) in distinct regions on a given nucleic acid molecule.

The minimal size of a nucleic acid molecule capable of forming a stable hybrid with a gene encoding an immunogenic T. gondii protein is typically at least about 12 to about 15 nucleotides in length if the nucleic acid molecule is GC-rich and at least about 15 to about 17 bases in length if it is AT-rich. The minimal size of a nucleic acid molecule used to encode an immunogenic T. gondii protein homolog of the present invention is from about 12 to about 18 nucleotides in length. Thus, the minimal size of an immunogenic T. gondii protein homolog of the present invention is from about 4 to about 6 amino acids in length. There is no limit, other than a practical limit, on the maximal size of a nucleic acid molecule encoding an immunogenic T. gondii protein of the present invention because a nucleic acid molecule of the present invention can include a portion of a gene, an entire gene, or multiple genes. A preferred nucleic acid molecule of the present invention is a nucleic acid molecule that is at least 12 nucleotides in length. Also preferred are nucleic acid molecules that are at least 18 nucleotides, or at least 20 nucleotides, or at least 25 nucleotides, or at least 30 nucleotides, or at least 40 nucleotides, or at least 50 nucleotides, or at least 70 nucleotides, or at least 100 nucleotides, or at least 150 nucleotides, or at least 200 nucleotides, or at least 250 nucleotides, or at least 300 nucleotides, or at least 350 nucleotides, or at least 400 nucleotides, or at least 500 nucleotides, or at least 750

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nucleotides, or at least 1000 nucleotides, or at least 1500 nucleotides, or at least 1750 nucleotides, or at least 2000 nucleotides, or at least 2250 nucleotides, or at least 2417 nucleotides in length, The preferred size of a protein encoded by a nucleic acid molecule of the present invention depends on whether a full-length, fusion, multivalent, or functional portion of such a protein is desired.

Stringent hybridization conditions are determined based on defined physical properties of the gene to which the nucleic acid molecule is being hybridized, and can be defined mathematically. Stringent hybridization conditions are those experimental parameters that allow an individual skilled in the art to identify significant similarities between heterologous nucleic acid molecules. These conditions are well known to those skilled in the art. See, for example, Sambrook, et al., 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Labs Press, and Meinkoth, et al., 1984, Anal. Biochem. 138, 267-284, each of which is incorporated by reference herein in its entirety. As explained in detail in the cited references, the determination of hybridization conditions involves the manipulation of a set of variables including the ionic strength (M, in moles/liter), the hybridization temperature (°C), the concentration of nucleic acid helix destabilizing agents (such as formamide), the average length of the shortest hybrid duplex (n), and the percent G + C composition of the fragment to which an unknown nucleic acid molecule is being hybridized. For nucleic acid molecules of at least about 150 nucleotides, these variables are inserted into a standard mathematical formula to calculate the melting temperature, or T_m, of a given nucleic acid molecule. As defined in the formula below, $T_{\rm m}$ is the temperature at which two complementary nucleic acid molecule strands will disassociate, assuming 100% complementarity between the two strands:

 T_m =81.5°C + 16.6 log M + 0.41(%G + C) - 500/n - 0.61(%formamide). For nucleic acid molecules smaller than about 50 nucleotides, hybrid stability is defined by the dissociation temperature (T_d), which is defined as the temperature at which 50% of the duplexes dissociate. For these smaller molecules, the stability at a standard ionic strength is defined by the following equation:

$$T_{d} = 4(G+C) + 2(A+T).$$

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A temperature of 5°C below T_d is used to detect hybridization between perfectly matched molecules.

Also well known to those skilled in the art is how base-pair mismatch, i.e. differences between two nucleic acid molecules being compared, including noncomplementarity of bases at a given location, and gaps due to insertion or deletion of one or more bases at a given location on either of the nucleic acid molecules being compared, will affect T_m or T_d for nucleic acid molecules of different sizes. For example, T_m decreases about 1°C for each 1% of mismatched base-pairs for hybrids greater than about 150 bp, and T_d decreases about 5°C for each mismatched base-pair for hybrids below about 50 bp. Conditions for hybrids between about 50 and about 150 base-pairs can be determined empirically and without undue experimentation using standard laboratory procedures well known to those skilled in the art. These simple procedures allow one skilled in the art to set the hybridization conditions (by altering, for example, the salt concentration, the formamide concentration or the temperature) so that only nucleic acid hybrids with less than a specified % base-pair mismatch will hybridize. Stringent hybridization conditions are commonly understood by those skilled in the art to be those experimental conditions that will allow hybridization between molecules having about 30% or less base-pair mismatch (i.e., about 70% or greater identity). Because one skilled in the art can easily determine whether a given nucleic acid molecule to be tested is less than or greater than about 50 nucleotides, and can therefore choose the appropriate formula for determining hybridization conditions, he or she can determine whether the nucleic acid molecule will hybridize with a given gene under stringent hybridization conditions and similarly whether the nucleic acid molecule will hybridize under conditions designed to allow a desired amount of base pair mismatch.

Hybridization reactions are often carried out by attaching the nucleic acid molecule to be hybridized to a solid support such as a membrane, and then hybridizing with a labeled nucleic acid molecule, typically referred to as a probe, suspended in a hybridization solution. Examples of common hybridization reaction techniques include, but are not limited to, the well-known Southern and northern blotting procedures.

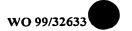
30 Typically, the actual hybridization reaction is done under non-stringent conditions, i.e.,

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at a lower temperature and/or a higher salt concentration, and then high stringency is achieved by washing the membrane in a solution with a higher temperature and/or lower salt concentration in order to achieve the desired stringency.

For example, if the skilled artisan wished to identify a nucleic acid molecule that hybridizes under stringent hybridization conditions with a *T. gondii* nucleic acid molecule of about 150 bp in length, the following conditions could preferably be used. As an example, the average G + C content of *Dirofilaria immitis* DNA is about 35%. The unknown nucleic acid molecules would be attached to a support membrane, and the 150 bp probe would be labeled, e.g. with a radioactive tag. The hybridization reaction could be carried out in a solution comprising 2X SSC and 0% formamide, at a temperature of about 37°C (low stringency conditions). Solutions of differing concentrations of SSC can be made by one of skill in the art by diluting a stock solution of 20X SSC (175.3 gram NaCl and about 88.2 gram sodium citrate in 1 liter of water, pH 7) to obtain the desired concentration of SSC. In order to achieve high stringency hybridization, the skilled artisan would calculate the washing conditions required to allow up to 30% base-pair mismatch. For example, in a wash solution comprising 1X SSC and 0% formamide, the T_m of perfect hybrids would be about 79°C:

 $81.5^{\circ}\text{C} + 16.6 \log (.15\text{M}) + (0.41 \times 35) - (500/150) - (0.61 \times 0) = 79^{\circ}\text{C}.$

Thus, to achieve hybridization with nucleic acid molecules having about 30% base-pair mismatch, hybridization washes would be carried out at a temperature of about 49°C. It is thus within the skill of one in the art to calculate additional hybridization temperatures based on the desired percentage base-pair mismatch, formulae and G/C content disclosed herein. For example, it is appreciated by one skilled in the art that as the nucleic acid molecule to be tested for hybridization against nucleic acid molecules of the present invention having sequences specified herein becomes longer than 150 nucleotides, the T_m for a hybridization reaction allowing up to 30% base-pair mismatch will not vary significantly from 49°C.

Furthermore, it is known in the art that there are commercially available computer programs for determining the degree of similarity between two nucleic acid sequences. These computer programs include various known methods to determine the

percentage identity and the number and length of gaps between hybrid nucleic acid molecules. Preferred methods to determine the percent identity among amino acid sequences and also among nucleic acid sequences include analysis using one or more of the commercially available computer programs designed to compare and analyze nucleic acid or amino acid sequences. These computer programs include, but are not limited to, GCGTM (available from Genetics Computer Group, Madison, WI), DNAsisTM (available from Hitachi Software, San Bruno, CA) and MacVectorTM (available from the Eastman Kodak Company, New Haven, CT). A preferred method to determine percent identity among amino acid sequences and also among nucleic acid sequences includes using the GCGTM program. Bestfit function with default parameter settings, or a gap weight of 12, a length weight of 4, an average match of 2.912, and an average mismatch of -2.003.

A preterred immunogenic *T. gondii* protein of the present invention is a compound that when administered to an animal in an effective manner, is capable of protecting that animal from disease caused by *T. gondii* or, in the case of cats, is capable of preventing *I. gondii* oocyst shedding in cats infected with *T. gondii*. In accordance with the present invention, the ability of an immunogenic *T. gondii* protein of the present invention to protect an animal from *T. gondii* disease refers to the ability of that protein to, for example, treat, ameliorate and/or prevent disease caused by *T. gondii*. In one embodiment, an immunogenic *T. gondii* protein of the present invention can elicit an immune response uncluding a humoral and/or cellular immune response) against *T. gondii*

The present invention also includes mimetopes of immunogenic *T. gondii* proteins of the present invention. As used herein, a mimetope of an immunogenic *T. gondii* protein of the present invention refers to any compound that is able to mimic the activity of such an immunogenic *T. gondii* protein, often because the mimetope has a structure that mimics the particular *T. gondii* protein. Mimetopes can be, but are not limited to peptides that have been modified to decrease their susceptibility to degradation such as all-D retro peptides; anti-idiotypic and/or catalytic antibodies, or fragments thereof: non-proteinaceous immunogenic portions of an isolated protein (e.g., carbohydrate structures); and synthetic or natural organic molecules, including nucleic

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acids. Such mimetopes can be designed using computer-generated structures of proteins of the present invention. Mimetopes can also be obtained by generating random samples of molecules, such as oligonucleotides, peptides or other organic molecules, and screening such samples by affinity chromatography techniques using the corresponding binding partner.

One embodiment of an immunogenic T. gondii protein of the present invention is a fusion protein that includes an immunogenic T. gondii protein-containing domain attached to one or more fusion segments. Suitable fusion segments for use with the present invention include, but are not limited to, segments that can: enhance a protein's stability; act as an immunopotentiator to enhance an immune response against an 10 immunogenic T. gondii protein; and/or assist in purification of an immunogenic T. gondii protein (e.g., by affinity chromatography). A suitable fusion segment can be a domain of any size that has the desired function (e.g., imparts increased stability, imparts increased immunogenicity to a protein, and/or simplifies purification of a protein). Fusion segments can be joined to amino and/or carboxyl termini of the immunogenic T. 15 gondii protein-containing domain of the protein and can be susceptible to cleavage in order to enable straightforward recovery of an immunogenic T. gondii protein. Fusion proteins are preferably produced by culturing a recombinant cell transformed with a nucleic acid molecule that encodes a protein including the fusion segment attached to either the carboxyl and/or amino terminal end of an immunogenic T. gondii protein-20 containing domain. Preferred fusion segments include a metal binding domain (e.g., a poly-histidine segment); an immunoglobulin binding domain (e.g., Protein A; Protein G; T cell; B cell; Fc receptor or complement protein antibody-binding domains); a sugar binding domain (e.g., a maltose binding domain); and/or a "tag" domain (e.g., at least a portion of β -galactosidase, a strep tag peptide, a T7 tag peptide, a FlagTM peptide, or 25 other domains that can be purified using compounds that bind to the domain, such as monoclonal antibodies). More preferred fusion segments include metal binding domains, such as a poly-histidine segment; a maltose binding domain; a strep tag peptide, such as that available from Biometra in Tampa, FL; and an S10 peptide. 30

In another embodiment, an immunogenic *T. gondii* protein of the present invention also includes at least one additional protein segment that is capable of

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protecting an animal from one or more diseases. Such a multivalent protective protein can be produced, for example, by culturing a cell transformed with a nucleic acid molecule comprising two or more nucleic acid domains joined together in such a manner that the resulting nucleic acid molecule is expressed as a multivalent protective compound containing at least two protective compounds capable of protecting an animal from diseases caused, for example, by at least one infectious agent.

Examples of multivalent protective compounds include, but are not limited to, an immunogenic *T. gondii* protein of the present invention attached to one or more compounds protective against one or more other infectious agents, particularly an agent that infects cats. In another embodiment, one or more protective compounds can be included in a multivalent vaccine comprising an immunogenic *T. gondii* protein of the present invention and one or more other protective molecules as separate compounds.

A preferred isolated immunogenic *T. gondii* protein of the present invention includes a protein that is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene (i.e., with the non-coding strand which is a complement of the coding strand) comprising at least one of the nucleic acid molecules cited in Table 1. As such, also preferred is a protein that is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with the non-coding strand of a gene comprising at least one of the nucleic acid sequences cited in Table 1. More preferred is a protein encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with at least one of the cited nucleic acid molecules particularly since those nucleic acid molecules have been shown to encode proteins that selectively bind to antiserum that either was raised against *T. gondii* oocysts, bradyzoites, or infected cat gut, or was isolated from a cat immune to *T. gondii* infection. As such, also preferred is a protein encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with the complement of at least one of the cited nucleic acid sequences.

Even more preferred are isolated proteins having an amino acid sequence encoded by a nucleic acid molecules that are at least about 75%, preferably at least about 80%, more preferably at least about 85%, even more preferably at least about 90%, even more preferably at least about 95%, and even more preferably at least about 98%

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identical to one of the nucleic acid molecules and/or nucleic acid sequences cited in Table 1. Also preferred are proteins that comprise one or more epitopes of any of the proteins having such amino acid sequences.

A particularly preferred isolated protein of the present invention is a protein having an amino acid sequence encoded by at least one of the cited nucleic acid molecules and or cited nucleic acid sequences, a protein encoded by an allelic variant of at least one of the cited nucleic acid molecules and/or nucleic acid sequences, or a protein comprising an epitope of any of the proteins having such amino acid sequences.

In one embodiment, preferred immunogenic *T. gondii* proteins of the present invention include proteins that are at least about 75%, preferably at least about 80%, more preferably at least about 85%, even more preferably at least about 90%, and even more preferably at least about 95% identical to at least one of the proteins cited in Table 1. As such, also preferred are proteins that are at least about 75%, preferably at least about 80%, even more preferably at least about 90%, and even more preferably at least about 95% identical to at least one of the amino acid sequences cited in Table 1. Also preferred are proteins that comprise one or more epitopes of any of such proteins. More preferred are immunogenic *T. gondii* proteins comprising the cited proteins and/or having the cited amino acid sequences, proteins encoded by allelic variants of nucleic acid molecules encoding proteins including the cited proteins and or having the cited amino acid sequences, and proteins having one or more epitopes of such proteins.

compressed a foodii nucleic acid molecule that encodes an immunogenic T. gondii protein. The identifying characteristics of such nucleic acid molecules are heretofore described. A nucleic acid molecule of the present invention can include an isolated natural foodin nucleic acid molecule or a homolog thereof, the latter of which is described in more detail below. A nucleic acid molecule of the present invention can include one or more regulatory regions, full-length or partial coding regions, or combinations thereof. The minimal size of a nucleic acid molecule of the present invention is a size sufficient to allow the formation of a stable hybrid (i.e., hybridization under stringent hybridization conditions) with the complementary sequence of another

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nucleic acid molecule. The minimal size of an *T. gondii* nucleic acid molecule of the present invention is from about 12 to about 18 nucleotides in length.

In accordance with the present invention, an isolated nucleic acid molecule is a nucleic acid molecule that has been removed from its natural milieu (i.e., that has been subjected to human manipulation) and can include DNA, RNA, or derivatives of either DNA or RNA. Accordingly, the term "isolated", as used herein to describe a nucleic acid molecule, does not reflect the extent to which the nucleic acid molecule has been purified. An isolated *T. gondii* nucleic acid molecule of the present invention can be isolated from its natural source or produced using recombinant nucleic acid technology (e.g., polymerase chain reaction (PCR) amplification or cloning) or chemical synthesis. Isolated *T. gondii* nucleic acid molecules can include, for example, natural allelic variants and nucleic acid molecules modified by nucleotide insertions, deletions, substitutions, and/or inversions in a manner such that the modifications do not substantially interfere with the nucleic acid molecule's ability to encode an immunogenic *T. gondii* protein of the present invention.

A homolog of a nucleic acid molecule encoding an immunogenic *T. gondii* protein can be produced using a number of methods known to those skilled in the art, see, for example, Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press; Sambrook et al., *ibid.*, is incorporated by reference herein in its entirety. For example, nucleic acid molecules can be modified using a variety of techniques including, but not limited to, classic mutagenesis and recombinant DNA techniques such as site-directed mutagenesis, chemical treatment, restriction enzyme cleavage, ligation of nucleic acid fragments, PCR amplification, synthesis of oligonucleotide mixtures and ligation of mixture groups to "build" a mixture of nucleic acid molecules, and combinations thereof. Nucleic acid molecule homologs can be selected by hybridization with a nucleic acid molecule encoding an immunogenic *T. gondii* protein or by screening the function of a protein encoded by the nucleic acid molecule (e.g., ability to elicit an immune response against at least one epitope of an immunogenic *T. gondii* protein).

An isolated nucleic acid molecule of the present invention can include a nucleic acid sequence that encodes at least one immunogenic *T. gondii* protein of the present

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invention, examples of which are disclosed herein. Although the phrase "nucleic acid molecule" primarily refers to the physical nucleic acid molecule and the phrase "nucleic acid sequence" primarily refers to the sequence of nucleotides on the nucleic acid molecule, the two phrases can be used interchangeably, especially with respect to a nucleic acid molecule, or a nucleic acid sequence, capable of encoding an *T. gondii* protein.

A preferred nucleic acid molecule of the present invention, when administered to a cat, is capable of preventing *T. gondii* oocyst shedding. As will be disclosed in more detail below, such a nucleic acid molecule can be, or encode, an antisense RNA, a molecule capable of triple helix formation, a ribozyme, or other nucleic acid-based drug compound. In additional embodiments, a nucleic acid molecule of the present invention can encode a protective protein (e.g., an immunogenic *T. gondii* protein of the present invention), the nucleic acid molecule being delivered to the animal, for example, by direct injection (i.e, as a genetic vaccine) or in a vehicle such as a recombinant virus vaccine or a recombinant cell vaccine. Another preferred nucleic acid molecule of the present invention, when administered to an animal, is capable of preventing disease in that animal caused by *T. gondii*.

One embodiment of the present invention is an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a nucleic acid molecule comprising at least one of the nucleic acid molecules cited in Table 1. As such, also preferred is a nucleic acid molecule that hybridizes under stringent hybridization conditions with at least one of the nucleic acid sequences cited in Table 1 or with a complement of such a sequence. More preferred is a nucleic acid molecule that hybridizes under stringent hybridization conditions with at least one of the cited nucleic acid molecules. As such, also preferred is a nucleic acid molecule that hybridizes under stringent hybridization conditions with at least one of the cited nucleic acid sequences or with a complement thereof.

Even more preferred are isolated nucleic acid molecules that are at least about 75%, preferably at least about 80%, more preferably at least about 85%, even more preferably at least about 90%, even more preferably at least about 95%, and even more preferably at least about 98% identical to one of the nucleic acid molecules and/or

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nucleic acid sequences cited in Table 1. Also preferred are nucleic acid molecules that form stable hybrids with nucleic acid molecules having those percent identities.

A particularly preferred isolated nucleic acid molecule of the present invention is a nucleic acid molecule that comprises at least one of the cited nucleic acid molecules and/or cited nucleic acid sequences, a nucleic acid molecule that is an allelic variant of at least one of the cited nucleic acid molecules and/or nucleic acid sequences, or a nucleic acid molecule that is a portion thereof (i.e., a nucleic acid molecule that forms a stable hybrid with at least one of the cited nucleic acid molecules or allelic variants thereof).

In one embodiment, a nucleic acid molecule encoding an immunogenic *T. gondii* protein of the present invention encodes a protein that is at least about 75%, preferably at least about 80%, more preferably at least about 85%, even more preferably at least about 90%, and even more preferably at least about 95% identical to the proteins cited in Table 1. Even more preferred is a nucleic acid molecule encoding a protein cited in Table 1 or an allelic variant of such a nucleic acid molecule. Also preferred are nucleic acid molecules encoding proteins comprising one or more epitopes of proteins having the cited percent identities or epitopes of proteins cited in Table 1 or encoded by nucleic acid molecules that are allelic variants of nucleic acid molecules cited in Table 1.

In another embodiment, a nucleic acid molecule encoding an immunogenic *T. gondii* protein of the present invention encodes a protein having an amino acid sequence that is at least about 75%, preferably at least about 80%, more preferably at least about 85%, even more preferably at least about 90%, and even more preferably at least about 95% identical to at least one of the amino acid sequences cited in Table 1. Even more preferred is a nucleic acid molecule encoding a protein having an amino acid sequence cited in Table 1 or an allelic variant of such a nucleic acid molecule. Also preferred are nucleic acid molecules encoding proteins comprising one or more epitopes of proteins having the cited percent identities or epitopes of proteins having amino acid sequences cited in Table 1 or encoded by nucleic acid molecules that are allelic variants of nucleic acid molecules cited in Table 1.

Note that nucleic acid molecules of the present invention can include nucleotide sequences in addition to those disclosed above, such as, but not limited to, nucleotide sequences comprising a full-length gene, a full-length coding region, a nucleic acid

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molecule encoding a fusion protein, or a nucleic acid molecule encoding a multivalent protective compound. Also included in the present invention are nucleic acid molecules that have been modified to accommodate codon usage properties of the cells in which such nucleic acid molecules are to be expressed. Preferred nucleic acid molecules of the present invention include fragments of the nucleic acid molecules disclosed in Table 1.

Knowing the nucleic acid sequences of certain nucleic acid molecules encoding immunogenic T. gondii proteins of the present invention allows one skilled in the art to, for example, (a) make copies of those nucleic acid molecules, (b) obtain nucleic acid molecules including at least a portion of such nucleic acid molecules (e.g., nucleic acid molecules including full-length genes, full-length coding regions, regulatory control sequences, truncated coding regions), and (c) obtain other nucleic acid molecules encoding an immunogenic T. gondii proteins. Such nucleic acid molecules can be obtained in a variety of ways including screening appropriate expression libraries with antibodies of the present invention; traditional cloning techniques using oligonucleotide probes of the present invention to screen appropriate libraries; and PCR amplification of appropriate libraries or DNA using oligonucleotide primers of the present invention. Preferred libraries to screen or from which to amplify nucleic acid molecules include T. gondii cDNA libraries as well as genomic DNA libraries. Similarly, preferred DNA sources from which to amplify nucleic acid molecules include T. gondii cDNA and genomic DNA. Techniques to clone and amplify nucleic acid molecules are disclosed, for example, in Sambrook et al., ibid.

The present invention also includes nucleic acid molecules that are oligonucleotides capable of hybridizing, under stringent hybridization conditions, with complementary regions of other, preferably longer, nucleic acid molecules of the present invention such as those comprising nucleic acid molecules encoding immunogenic T. gondii proteins. Oligonucleotides of the present invention can be RNA, DNA, or derivatives of either. The minimum size of such oligonucleotides is the size required for formation of a stable hybrid between an oligonucleotide and a complementary sequence on a nucleic acid molecule of the present invention. A preferred oligonucleotide of the present invention has a maximum size of about 100 nucleotides. The present invention includes oligonucleotides that can be used as, for example, probes to identify nucleic

acid molecules encoding immunogenic *T. gondii* proteins, primers to produce nucleic acid molecules encoding immunogenic *T. gondii* proteins, or reagents to inhibit immunogenic *T. gondii* protein production or activity (e.g., as antisense-, triplex formation-, ribozyme- and/or RNA drug-based reagents). The present invention also includes the use of such oligonucleotides to protect animals from disease using one or more of such technologies. Appropriate oligonucleotide-containing compositions can be administered to an animal using techniques known to those skilled in the art.

One embodiment of the present invention includes a recombinant vector, which includes at least one isolated nucleic acid molecule of the present invention, inserted into any vector capable of delivering the nucleic acid molecule into a host cell. Such a vector contains heterologous nucleic acid sequences, that is nucleic acid sequences that are not naturally found adjacent to nucleic acid molecules of the present invention and that preferably are derived from a species other than the species from which the nucleic acid molecule(s) are derived. The vector can be either RNA or DNA, either prokaryotic or eukaryotic, and typically is a virus or a plasmid. Recombinant vectors can be used in the cloning, sequencing, and/or otherwise manipulating of nucleic acid molecule encoding immunogenic *T. gondii* proteins of the present invention.

One type of recombinant vector, referred to herein as a recombinant molecule, comprises a nucleic acid molecule of the present invention operatively linked to an expression vector. The phrase operatively linked refers to insertion of a nucleic acid molecule into an expression vector in a manner such that the molecule is able to be expressed when transformed into a host cell. As used herein, an expression vector is a DNA or RNA vector that is capable of transforming a host cell and of effecting expression of a specified nucleic acid molecule. Preferably, the expression vector is also capable of replicating within the host cell. Expression vectors can be operative in either prokaryotic or eukaryotic cells, and are typically viruses or plasmids. Expression vectors of the present invention include any vectors that function (i.e., direct gene expression) in recombinant cells of the present invention, including in bacterial, fungal, parasite, insect, other animal, and plant cells. Preferred expression vectors of the present invention can direct gene expression in bacterial, yeast, *T. gondii* and mammalian cells, and more preferably in the cell types disclosed herein.

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In particular, expression vectors of the present invention contain regulatory sequences such as transcription control sequences, translation control sequences, origins of replication, and other regulatory sequences that are compatible with the recombinant cell and that control the expression of nucleic acid molecules of the present invention. In particular, recombinant molecules of the present invention include transcription control sequences. Transcription control sequences are sequences which control the initiation, elongation, and termination of transcription. Particularly important transcription control sequences are those which control transcription initiation, such as promoter, enhancer, operator and repressor sequences. Suitable transcription control sequences include any transcription control sequence that can function in at least one of the recombinant cells of the present invention. A variety of such transcription control sequences are known to those skilled in the art. Preferred transcription control sequences include those which function in bacterial, yeast, helminth or other endoparasite, or insect and mammalian cells, such as, but not limited to, tac, lac, trp, trc, oxy-pro, omp/lpp, rrnB, bacteriophage lambda (such as lambda p_L and lambda p_R and fusions that include such promoters), bacteriophage T7, T7lac, bacteriophage T3, bacteriophage SP6, bacteriophage SP01, metallothionein, alpha-mating factor, Pichia alcohol oxidase, alphavirus subgenomic promoter, antibiotic resistance gene, baculovirus, Heliothis zea insect virus, vaccinia virus, herpesvirus, raccoon poxvirus, other poxvirus, adenovirus, cytomegalovirus (such as immediate early promoter), simian virus 40, retrovirus, actin, retroviral long terminal repeat, Rous sarcoma virus, heat shock, phosphate and nitrate transcription control sequences as well as other sequences capable of controlling gene expression in prokaryotic or eukaryotic cells. Additional suitable transcription control sequences include tissue-specific promoters and enhancers as well as lymphokine-inducible promoters (e.g., promoters inducible by interferons or interleukins). Transcription control sequences of the present invention can also include naturally occurring transcription control sequences naturally associated with T. gondii.

Suitable and preferred nucleic acid molecules to include in recombinant vectors of the present invention are as disclosed herein. Preferred nucleic acid molecules to include in recombinant vectors, and particularly in recombinant molecules, include those cited in Table 1. Particularly preferred recombinant molecules of the present invention

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include those recombinant molecules, the production of which are described in the Examples section.

Recombinant molecules of the present invention may also (a) contain secretory signals (i.e., signal segment nucleic acid sequences) to enable an expressed *T. gondii* protein of the present invention to be secreted from the cell that produces the present and/or (b) contain fusion sequences which lead to the expression of nucleic acid molecules of the present invention as fusion proteins. Examples of suitable signal segments include any signal segment capable of directing the secretion of a protein of the present invention. Preferred signal segments include, but are not limited to, tissue plasminogen activator (t-PA), interferon, interleukin, growth hormone, histocompatibility and viral envelope glycoprotein signal segments. Suitable fusion segments encoded by fusion segment nucleic acids are disclosed herein. In addition, a nucleic acid molecule of the present invention can be joined to a fusion segment that directs the encoded protein to the proteosome, such as a ubiquitin fusion segment. Eukaryotic recombinant molecules may also include intervening and/or untranslated sequences surrounding and/or within the nucleic acid sequences of nucleic acid molecules of the present invention.

Another embodiment of the present invention includes a recombinant cell comprising a host cell transformed with one or more recombinant molecules of the 20 present invention. Transformation of a nucleic acid molecule into a cell can be accomplished by any method by which a nucleic acid molecule can be inserted into the cell. Transformation techniques include, but are not limited to, transfection, electroporation, microinjection, lipofection, adsorption, and protoplast fusion. A recombinant cell may remain unicellular or may grow into a tissue, organ or a multicellular organism. Transformed nucleic acid molecules of the present invention can 25 remain extrachromosomal or can integrate into one or more sites within a chromosome of the transformed (i.e., recombinant) cell in such a manner that their ability to be expressed is retained. Preferred nucleic acid molecules with which to transform a cell include nucleic acid molecules encoding immunogenic T. gondii proteins disclosed 30 herein. Particularly preferred nucleic acid molecules with which to transform a cell include those listed in Table 1.

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Suitable host cells to transform include any cell that can be transformed with a nucleic acid molecule of the present invention. Host cells can be either untransformed cells or cells that are already transformed with at least one nucleic acid molecule (e.g., nucleic acid molecules encoding one or more proteins of the present invention and/or other proteins useful in the production of multivalent vaccines). Host cells of the present invention either can be endogenously (i.e., naturally) capable of producing T. gondii proteins of the present invention or can be capable of producing such proteins after being transformed with at least one nucleic acid molecule of the present invention. Host cells of the present invention can be any cell capable of producing at least one protein of the present invention, and include bacterial, fungal (including yeast), parasite (including helminth, protozoa and ectoparasite), insect, other animal and plant cells. Preferred host cells include bacterial, mycobacterial, yeast, protozoan, helminth, insect and mammalian cells. More preferred host cells include Salmonella, Escherichia, Bacillus, Listeria, Saccharomyces, Spodoptera, Mycobacteria, Trichoplusia, BHK (baby hamster kidney) cells, MDCK cells (Madin-Darby canine kidney cell line), CRFK cells (Crandell feline kidney cell line), CV-1 cells (African monkey kidney cell line used, for example, to culture raccoon poxvirus), COS (e.g., COS-7) cells, and Vero cells. Particularly preferred host cells are Escherichia coli, including E. coli K-12 derivatives; Salmonella typhi; Salmonella typhimurium, including attenuated strains such as UK-1 $_{\chi}3987$ and SR-11 x4072; Spodoptera frugiperda; Trichoplusia ni; BHK cells; MDCK cells; CRFK 20 cells; CV-1 cells; COS cells; Vero cells; and non-tumorigenic mouse myoblast G8 cells (e.g., ATCC CRL 1246). Additional appropriate mammalian cell hosts include other kidney cell lines, other fibroblast cell lines (e.g., human, murine or chicken embryo fibroblast cell lines), myeloma cell lines, Chinese hamster ovary cells, mouse NIH/3T3 cells, LMTK31 cells and/or HeLa cells. In one embodiment, the proteins may be 25 expressed as heterologous proteins in myeloma cell lines employing immunoglobulin promoters.

A recombinant cell is preferably produced by transforming a host cell with one or more recombinant molecules, each comprising one or more nucleic acid molecules of the present invention operatively linked to an expression vector containing one or more transcription control sequences, examples of which are disclosed herein.

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A recombinant cell of the present invention includes any cell transformed with at least one of any nucleic acid molecule of the present invention. Suitable and preferred nucleic acid molecules as well as suitable and preferred recombinant molecules with which to transfer cells are disclosed herein. Particularly preferred recombinant cells include those recombinant cells, the production of which are disclosed in the Examples section.

Recombinant cells of the present invention can also be co-transformed with one or more recombinant molecules including a nucleic acid molecule encoding at least one immunogenic *T* gondii protein of the present invention and one or more other nucleic acid molecules encoding other protective compounds, as disclosed herein (e.g., to produce multivalent vaccines).

Recombinant DNA technologies can be used to improve expression of transformed nucleic acid molecules by manipulating, for example, the number of copies of the nucleic and molecules within a host cell, the efficiency with which those nucleic acid molecules are transcribed, the efficiency with which the resultant transcripts are translated, and the efficiency of post-translational modifications. Recombinant techniques useful for increasing the expression of nucleic acid molecules of the present invention include, but are not limited to, operatively linking nucleic acid molecules to high-copy number plasmids, integration of the nucleic acid molecules into one or more host cell chromosomes, addition of vector stability sequences to plasmids, substitutions or models attended transcription control signals (e.g., promoters, operators, enhancers), substitutions or modifications of translational control signals (e.g., ribosome binding sites. Shim the carno sequences), modification of nucleic acid molecules of the present invention to a respond to the codon usage of the host cell, deletion of sequences that destabilize transcripts, and use of control signals that temporally separate recombinant cell growth tram recombinant enzyme production during fermentation. The activity of an expressed recombinant protein of the present invention may be improved by fragmenting, modifying, or derivatizing nucleic acid molecules encoding such a protein.

Isolated I gondii proteins of the present invention can be produced in a variety of ways, including production and recovery of natural proteins, production and recovery of recombinant proteins, and chemical synthesis of the proteins. In one embodiment, an

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isolated protein of the present invention is produced by culturing a cell capable of expressing the protein under conditions effective to produce the protein, and recovering the protein. A preferred cell to culture is a recombinant cell of the present invention. Effective culture conditions include, but are not limited to, effective media, bioreactor, temperature, pH and oxygen conditions that permit protein production. An effective medium refers to any medium in which a cell is cultured to produce an immunogenic *T. gondii* protein of the present invention. Effective media typically comprise an aqueous medium having assimilable carbon, nitrogen and phosphate sources, and appropriate salts, minerals, metals and other nutrients, such as vitamins. Cells of the present invention can be cultured in conventional fermentation bioreactors, shake flasks, test tubes, microtiter dishes, and petri plates. Culturing can be carried out at a temperature, pH and oxygen content appropriate for a recombinant cell. Suitable culturing conditions are within the expertise of one of ordinary skill in the art. Examples of suitable conditions are included in the Examples section.

Depending on the vector and host system used for production, resultant proteins of the present invention may either remain within the recombinant cell; be secreted into the fermentation medium; be secreted into a space between two cellular membranes, such as the periplasmic space in *E. coli*; or be retained on the outer surface of a cell or viral membrane.

The phrase "recovering the protein", as well as similar phrases, refers to collecting the whole fermentation medium containing the protein and need not imply additional steps of separation or purification. Proteins of the present invention can be purified using a variety of standard protein purification techniques, such as, but not limited to, affinity chromatography, ion exchange chromatography, filtration, electrophoresis, hydrophobic interaction chromatography, gel filtration chromatography, reverse phase chromatography, concanavalin A chromatography, chromatofocusing and differential solubilization. Proteins of the present invention are preferably retrieved in "substantially pure" form. As used herein, "substantially pure" refers to a purity that allows for the effective use of the protein as a composition to inhibit *T. gondii* oocyst shedding in a cat due to infection with *T. gondii*, or for preventing *T. gondii* infection in an animal, or as a diagnostic reagent. A composition for inhibiting *T. gondii* oocyst

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shedding in a cat due to infection with *T. gondii* animals, or for preventing *T. gondii* infection in an animal for example, should exhibit no substantial toxicity and preferably should be capable of stimulating the production of antibodies in a treated animal.

The present invention also includes isolated (i.e., removed from their natural milieu) antibodies that selectively bind to an immunogenic *T. gondii* protein of the present invention or a mimetope thereof (e.g., anti-*T. gondii* antibodies). As used herein, the term "selectively binds to" an immunogenic *T. gondii* protein refers to the ability of antibodies of the present invention to preferentially bind to specified proteins and mimetopes thereof of the present invention. Binding can be measured using a variety of methods standard in the art including enzyme immunoassays (e.g., ELISA), immunoblot assays, etc.; see, for example, Sambrook et al., *ibid.*, and Harlow, et al., 1988, *Antibodies, a Laboratory Manual*, Cold Spring Harbor Labs Press; Harlow et al., *ibid.*, is incorporated by reference herein in its entirety. An anti-*T. gondii* antibody of the present invention preferably selectively binds to an immunogenic *T. gondii* protein in such a way as to inhibit the function of that protein.

Isolated antibodies of the present invention can include antibodies in any bodily fluid that has been collected (e.g., recovered) from an animal. Suitable bodily fluids include, but are not limited to, blood, serum, plasma, urine, tears, aqueous humor, central nervous system fluid (CNF), saliva, lymph, nasal secretions, milk and feces. Thus, serum containing antibodies (i.e., antiserum) or mucosal secretions, such as intestinal secretions, are examples of isolated antibodies. Other embodiments of antibodies include antibodies that have been purified to varying degrees. Antibodies of the present invention can be polyclonal or monoclonal, or can be functional equivalents such as antibody fragments and genetically-engineered antibodies, including single chain antibodies or chimeric antibodies that can bind to one or more epitopes.

A preferred method to produce antibodies of the present invention includes (a) administering to an animal an effective amount of a protein, peptide or mimetope thereof of the present invention to produce the antibodies and (b) recovering the antibodies. In another method, antibodies of the present invention are produced recombinantly using techniques as heretofore disclosed to produce *T. gondii* proteins of the present invention.

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Antibodies raised against defined proteins or mimetopes can be advantageous because such antibodies are not substantially contaminated with antibodies against other substances that might otherwise cause interference in a diagnostic assay or side effects if used in a composition for inhibiting *T. gondii* oocyst shedding in a cat due to infection with *T. gondii*, or for preventing *T. gondii* infection in an animal.

Antibodies of the present invention have a variety of potential uses that are within the scope of the present invention. For example, such antibodies can be used (a) as compounds to passively immunize a cat in order to inhibit the cat from shedding T. gondu occysts. (b) as reagents in assays to detect infection by T. gondii and/or (c) as tools to screen expression libraries and/or to recover desired proteins of the present invention from a mixture of proteins and other contaminants.

One embediment of the present invention includes a method for identifying a nucleic acid molecule encoding an immunogenic T. gondii protein. According to this method, antiserum (comprising either monoclonal or polyclonal antibodies) raised against a 1 gondu developmental stage or stages, or against oocysts, is used to immunoscreen a T. gondii genomic expression library or a T. gondii cDNA expression nucleic acid molecule expressing an immunogenic T. gondii protein is identified by its ability to selectively bind to at least one antibody within the antiserum. As used herein, the term immunoscreen refers to a method in which antibodies are mixed with a sample to determine whether the sample contains a substance to which the antibade can selectively bind. A substance is identified by its ability to selectively bind to the antibodies. Although general methods to accomplishing immunoscreening of expression libraries are known to those skilled in the art, the exact method to use such a technique to identify T. gondii immunogenic proteins was not previously known. The present invention includes the identification of antisera that are useful in the identification and isolation of nucleic acid molecules encoding T. gondii immunogenic protein. Nuch antisera include antiserum raised against T. gondii oocysts, antiserum raised against I gondii bradyzoites, antiserum raised against I. gondii infected cat gut, and antiscrum isolated from a cat immune to T. gondii infection. In one embodiment, antiscrum as described above is enriched for antibodies specific to T. gondii

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gametogenic stages. In a preferred embodiment, polyclonal antiserum is produced by exposing an animal to a *T. gondii* antigen or antigens, then isolating the antiserum from the animal so exposed. Methods to produce and use the various antisera are described in the Examples section.

In another embodiment, immunoscreening as described above can be used to identify an immunogenic *T. gondii* protein. According to this method, antiserum as described above is used to immunoscreen a *T. gondii* genomic expression library or cDNA expression library, and an immunogenic T. gondii protein is identified. *T. gondii* immunogenic proteins can also be identified by immunoscreening preparations containing *T. gondii* antigens (e.g., *T. gondii* oocysts, bradyzoites, infected cat guts) using antiserum as described above.

Nucleic acid molecules and proteins identified using such techniques can be isolated (i.e., recovered) and purified to a desired state of purity using techniques known to those skilled in the art.

One embodiment of the present invention is a composition that, when administered to a cat in an effective manner, is capable of preventing that cat from shedding T. gondii oocysts. Compositions of the present invention, useful for inhibiting T. gondii oocyst shedding in a cat due to infection with T. gondii (i.e., infection with T. gondii causes oocyst shedding in cats), include at least one of the following protective compounds: an isolated immunogenic T. gondii protein or a mimetope thereof, an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a nucleic acid molecule comprising one of the nucleic acid molecules and/or nucleic acid sequences cited in Table 1, an isolated antibody that selectively binds to an immunogenic T. gondii protein, an inhibitor of T. gondii function identified by its ability to bind to an immunogenic T. gondii protein and thereby impede development and/or the production of oocysts, or a mixture thereof (i.e., combination of at least two of the compounds). As used herein, a protective compound refers to a compound that, when administered to a cat in an effective manner, is able to inhibit the cat from shedding T. gondii oocysts upon infection with T. gondii. The term protective compound also refers to a compound that, when administered to a cat or other animal, including a human, in

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an effective manner, is able to prevent or ameliorate disease caused by infection with T. gondii. Examples of proteins, nucleic acid molecules, antibodies and inhibitors of the present invention are disclosed herein.

The present invention also includes a composition comprising at least one T. gondii protein-based compound of the present invention in combination with at least one additional compound protective against one or more infectious agents. Examples of such compounds and infectious agents are disclosed herein.

Compositions of the present invention that are useful for preventing *T. gondii* infection can be administered to any animal susceptible to such therapy, preferably to mammals.

In order to inhibit a cat from shedding *T. gondii* oocysts, a composition of the present invention is administered to the cat in a manner effective to inhibit that cat from shedding *T. gondii* oocysts. In a preferred embodiment, compositions of the present invention are administered to cats prior to infection in order to prevent oocyst shedding (i.e., as a preventative vaccine). In another embodiment, compositions of the present invention can be administered to animals after infection in order to treat disease caused by *T. gondii* (e.g., as a therapeutic vaccine).

Compositions of the present invention, useful for inhibiting *T. gondii* oocyst shedding in a cat due to infection with *T. gondii*, or for preventing *T. gondii* infection in an animal, can be formulated in an excipient that the animal to be treated can tolerate. Examples of such excipients include water, saline, Ringer's solution, dextrose solution, Hank's solution, and other aqueous physiologically balanced salt solutions. Nonaqueous vehicles, such as fixed oils, sesame oil, ethyl oleate, or triglycerides may also be used. Other useful formulations include suspensions containing viscosity enhancing agents, such as sodium carboxymethylcellulose, sorbitol, or dextran. Excipients can also contain minor amounts of additives, such as substances that enhance isotonicity and chemical stability. Examples of buffers include phosphate buffer, bicarbonate buffer and Tris buffer, while examples of preservatives include thimerosal, — or o-cresol, formalin and benzyl alcohol. Standard formulations can either be liquid injectables or solids which can be taken up in a suitable liquid as a suspension or solution for injection. Thus, in a

non-liquid formulation, the excipient can comprise dextrose, human serum albumin, preservatives, etc., to which sterile water or saline can be added prior to administration.

In one embodiment of the present invention, a composition useful for inhibiting oocyst shedding in a cat infected with T. gondii, or for preventing T. gondii infection in an animal, can include an adjuvant. Adjuvants are agents that are capable of enhancing the immune response of an animal to a specific antigen. Suitable adjuvants include, but are not limited to, cytokines, chemokines, and compounds that induce the production of cytokines and chemokines (e.g., granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), colony stimulating factor (CSF), erythropoietin (EPO), interleukin 2 (IL-2), interleukin-3 (IL-3), interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 6 (IL-6), interleukin 7 (IL-7), interleukin 8 (IL-8), interleukin 10 (IL-10), interleukin 12 (IL-12), interferon gamma, interferon gamma inducing factor I (IGIF), transforming growth factor beta, RANTES (regulated upon activation, normal T-cell expressed and presumably secreted), macrophage inflammatory proteins (e.g., MIP-1 alpha and MIP-1 beta), and Leishmania elongation initiating factor (LEIF)); bacterial components (e.g., endotoxins, in particular superantigens, exotoxins and cell wall components); aluminumbased salts; calcium-based salts; silica; polynucleotides; toxoids; serum proteins, viral coat proteins; block copolymer adjuvants (e.g., Hunter's Titermax™ adjuvant (Vaxcel™, Inc. Norcross, GA), Ribi adjuvants (Ribi ImmunoChem Research, Inc., Hamilton, MT); and saponins and their derivatives (e.g., Quil A (Superfos Biosector A/S, Denmark). Protein adjuvants of the present invention can be delivered in the form of the protein themselves or of nucleic acid molecules encoding such proteins using the methods described herein.

In one embodiment of the present invention, a composition useful for inhibiting oocyst shedding in a cat infected with *T. gondii*, or for preventing *T. gondii* infection in an animal, can include a carrier. Carriers include compounds that increase the half-life of a composition of the present invention in the treated animal. Suitable carriers include, but are not limited to, polymeric controlled release vehicles, biodegradable implants, liposomes, bacteria, viruses, other cells, oils, esters, and glycols.

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One embodiment of the present invention is a controlled release formulation that is capable of slowly releasing a composition of the present invention into an animal. As used herein, a controlled release formulation comprises a composition of the present invention in a controlled release vehicle. Suitable controlled release vehicles include, but are not limited to, biocompatible polymers, other polymeric matrices, capsules, microcapsules, microparticles, bolus preparations, osmotic pumps, diffusion devices, liposomes, lipospheres, and transdermal delivery systems. Other controlled release formulations of the present invention include liquids that, upon administration to an animal, form a solid or a gel *in situ*. Preferred controlled release formulations are biodegradable (i.e., bioerodible).

A preferred controlled release formulation of the present invention is capable of releasing a composition of the present invention into the blood of the treated animal at a constant rate sufficient to attain dose levels of the composition effective to either inhibit oocyst shedding by cats, or to protect an animal from disease caused by *T. gondii*. The composition is preferably released over a period of time ranging from about 1 to about 12 months. A controlled release formulation of the present invention is capable of effecting a treatment preferably for at least about 1 month, more preferably for at least about 3 months, even more preferably for at least about 6 months, even more preferably for at least about 12 months.

Compositions of the present invention can be administered to cats prior to infection in order to inhibit oocyst shedding, and/or can be administered to cats or other animals, including humans, before infection in order to prevent disease caused by T. gondii infection, or after infection in order to treat disease caused by T. gondii. For example, nucleic acid molecules, proteins, mimetopes thereof, antibodies thereof, and inhibitors thereof can be used to treat or prevent disease caused by T. gondii infection. Acceptable protocols to administer compositions of the present invention include individual dose size, number of doses, frequency of dose administration, and mode of administration. Determination of such protocols can be accomplished by those skilled in the art. A suitable single dose is a dose that is capable of protecting an animal from disease when administered one or more times over a suitable time period. For example, a preferred single dose of a protein, mimetope or antibody composition of the present

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invention is from about 1 microgram (µg) to about 10 milligrams (mg) of the composition per kilogram body weight of the animal. Booster doses can be administered from about 2 weeks to several years after the original administration. Booster administrations preferably are administered when the immune response of the animal becomes insufficient to protect the animal from disease. A preferred administration schedule is one in which from about 10 µg to about 1 mg of the composition per kg body weight of the animal is administered from about one to about two times over a time period of from about 2 weeks to about 12 months. Modes of administration can include, but are not limited to, injection, oral administration, inhalation, nasal administration, intraocular administration, anal administration, topical administration, particle bombardment, and intradermal scarification. Preferred injection methods include intradermal, intramuscular, subcutaneous, intravenous methods, with intradermal injection and intramuscular injection being more preferred. A particularly preferred method is mucosal administration.

According to one embodiment, a nucleic acid molecule of the present invention can be administered to an animal in a fashion to enable expression of that nucleic acid molecule into a protective protein or protective RNA (e.g., antisense RNA, ribozyme, triple helix forms or RNA drug) in the animal. Nucleic acid molecules can be delivered to an animal in a variety of methods including, but not limited to, (a) administering a nucleic acid not packaged in a viral coat or cell as a genetic vaccine (e.g., as "naked" DNA or RNA molecules with or without a non-viral/non-cellular carrier (e.g., liposome, hydrogel, etc.) or (b) administering a nucleic acid molecule packaged as a recombinant virus vaccine or as a recombinant cell vaccine (i.e., the nucleic acid molecule is delivered by a viral or cellular vehicle).

A genetic vaccine of the present invention includes a recombinant molecule of the present invention. As such, a genetic vaccine comprises at least one isolated nucleic acid molecule encoding an immunogenic *T. gondii* protein operatively linked to a eukaryotic or prokaryotic transcription control region. A genetic vaccine can be either RNA or DNA, can have components from prokaryotic as well as eukaryotic sources, and can have the ability, by methods described herein, to enter either eukaryotic or prokaryotic cells and direct expression of isolated nucleic acid molecules of the present

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invention in those cells. In a preferred embodiment, a genetic vaccine of the present invention includes a recombinant virus genome (i.e., a nucleic acid molecule of the present invention ligated to at least one viral genome in which transcription of the nucleic acid molecule is directed either by a transcription control region on the genome or a separate transcription control region) or a recombinant plasmid that includes a nucleic acid molecule of the present invention ligated into a vector that is not a viral genome such that the nucleic acid molecule is operatively linked to a transcription control region.

A genetic vaccine of the present invention includes a nucleic acid molecule of the present invention and preferably includes a recombinant molecule of the present invention that preferably is replication, or otherwise amplification, competent. A genetic vaccine of the present invention can comprise one or more nucleic acid molecules of the present invention in the form of, for example, a dicistronic recombinant molecule. Preferred genetic vaccines include at least a portion of a viral genome (i.e., a viral vector) and a nucleic acid molecule of the present invention. Preferred viral vectors include those based on alphaviruses, poxviruses, adenoviruses, adeno-associated viruses, herpesviruses, picornaviruses, and retroviruses, with those based on alphaviruses (e.g., Sindbis virus or Semliki forest virus), picornaviruses (e.g., poliovirus or mengovirus), species-specific herpesviruses and poxviruses being particularly preferred. Any suitable transcription control sequence can be used, including those disclosed as suitable for protein production. Particularly preferred transcription control sequences include cytomegalovirus immediate early (preferably in conjunction with Intron-A), Rous sarcoma virus long terminal repeat, and tissue-specific transcription control sequences, as well as transcription control sequences endogenous to viral vectors if viral vectors are used. The incorporation of a "strong" polyadenylation signal is also preferred.

Genetic vaccines of the present invention can be administered in a variety of ways, with intramuscular, subcutaneous, intradermal, transdermal, intraocular, intranasal and oral routes of administration being preferred. A preferred single dose of a genetic vaccine ranges from about 1 nanogram (ng) to about 600 µg, depending on the route of administration and/or method of delivery, as can be determined by those skilled in the art. Suitable delivery methods include, for example, by injection, by gene gun, as drops,

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as inhaled aerosols, ingested in microparticles or microcapsules, and/or topical delivery. Genetic vaccines of the present invention can be contained in an aqueous excipient (e.g., phosphate buffered saline) alone or in a carrier (e.g., lipid-based vehicles).

A recombinant virus vaccine of the present invention includes a recombinant molecule of the present invention that is packaged in a viral coat and that can be expressed in an animal after administration. Preferably, the recombinant molecule is packaging- or replication-deficient and/or encodes an attenuated virus. A number of recombinant viruses can be used, including, but not limited to, those based on alphaviruses, poxviruses, adenoviruses, herpesviruses, picornaviruses, and retroviruses. Preferred recombinant virus vaccines are those based on alphaviruses (e.g., Sindbis virus), picornaviruses (e.g., poliovirus, mengovirus), raccoon poxviruses, speciesspecific herpesviruses and species-specific poxviruses. An example of methods to produce and use alphavirus recombinant virus vaccines are disclosed in PCT Publication No. WO 94/17813, by Xiong et al., published August 18, 1994, which is incorporated by reference herein in its entirety.

When administered to an animal, a recombinant virus vaccine of the present invention infects cells within the immunized animal and directs the production of a protective protein or RNA nucleic acid molecule that is capable of preventing a cat from shedding oocysts as disclosed herein. For example, a recombinant virus vaccine comprising a nucleic acid molecule encoding an immunogenic *T. gondii* protein of the present invention is administered according to a protocol that results in the subject cat producing a sufficient immune response to inhibit shedding *T. gondii* oocysts. A preferred single dose of a recombinant virus vaccine of the present invention is from about 1 x 10⁴ to about 1 x 10⁸ virus plaque forming units (pfu) per kilogram body weight of the animal. Administration protocols are similar to those described herein for protein-based vaccines, with subcutaneous, intramuscular, intraocular, intranasal and oral administration routes being preferred.

A recombinant cell vaccine of the present invention includes recombinant cells of the present invention that express at least one protein of the present invention. Preferred recombinant cells for this embodiment include Salmonella, E. coli, Listeria,

Mycobacterium, S. frugiperda, yeast, (including Saccharomyces cerevisiae and Pichia

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pastoris), BHK, CV-1, myoblast G8, COS (e.g., COS-7), Vero, MDCK and CRFK recombinant cells. Recombinant cell vaccines of the present invention can be administered in a variety of ways but have the advantage that they can be administered orally, preferably at doses ranging from about 10⁸ to about 10¹² cells per kilogram body weight. Administration protocols are similar to those described herein for protein-based vaccines. Recombinant cell vaccines can comprise whole cells, cells stripped of cell walls or cell lysates.

The efficacy of a composition of the present invention to inhibit oocyst shedding caused by *T. gondii* can be tested in a variety of ways including, but not limited to, detection of protective antibodies (using, for example, proteins or mimetopes of the present invention), detection of cellular immunity within the treated animal, or challenge of the treated animal with *T. gondii* to determine whether the treated animal is resistant to oocyst shedding. Challenge studies can include direct administration of *T. gondii* tachyzoites or tissue cysts or sporulated oocysts (the infective stages) to the treated animal. In one embodiment, compositions of the present invention can be tested in animal models such as mice. Such techniques are known to those skilled in the art.

One preferred embodiment of the present invention is the use of immunogenic *T. gondii* proteins, nucleic acid molecules encoding immunogenic *T. gondii* proteins, antibodies and inhibitors of the present invention, to inhibit a cat from shedding oocysts. It is particularly preferred to prevent intestinal stages of the parasite from developing into oocysts. Preferred compositions are those that are able to inhibit at least one step in the portion of the parasite's development cycle that occurs in the intestines prior to the development of oocysts. In cats infected with tissue cysts, for example, the prepatent period for oocyst shedding is three to five days. When cats are infected with sporulated oocysts, for example, the prepatent period can range from 19 to 45 days. Particularly preferred compositions useful for inhibiting oocyst shedding in a cat infected with *T. gondii* include *T. gondii*-based compositions of the present invention. Such compositions include nucleic acid molecules encoding immunogenic *T. gondii* proteins, immunogenic *T. gondii* proteins and mimetopes thereof and anti-*T. gondii* antibodies. Compositions of the present invention are administered to cats in a manner effective to inhibit the cats from shedding *T. gondii* oocysts. Additional protection may be obtained

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by administering additional protective compounds, including other *T. gondii* proteins, nucleic acid molecules and antibodies, as disclosed herein.

It is also within the scope of the present invention to use isolated proteins, mimetopes, nucleic acid molecules and antibodies of the present invention as diagnostic reagents to detect infection by *T. gondii*. These diagnostic reagents can further be supplemented with additional compounds that can specifically detect any or all phases of the parasite's life cycle. General methods to use diagnostic reagents in the diagnosis of disease are known to those skilled in the art. A method or a kit for the detection of *T. gondii* infection could be combined with reagents for the detection of additional infectious agents, for example viruses (e.g. Coronaviruses), bacteria (e.g. *Campylobacter, Clostridium, Salmonella*), protozoa (e.g. *Cryptosporidium, Giardia, Isospora Hammondia, Sarcocystis, Besnoitia, Microsporidium*), and/or multi-cellular organisms (c.g. *Leama, Anclostoma, Toxocara, Physaloptera, Paragonimus, Strongyloides, Irichuris*).

15 Another embodiment of the present invention is a method to detect microscopic parasite cysts or excysts in feces using PCR amplification techniques. By microscopic, it is meant exists or oocysts that are too small to be conveniently detected by simple visual observation of the feces. Preferred organisms to be detected include oocysts from infectious protozoan parasites including members of the apicomplexa and others 20 including by example, Toxoplasma, Cryptosporidium, Isospora, Giardia, Eimeria, Hammond... Securistis, Besnoitia, Microsporidium. Additional infectious agents to detect include the example, viruses (e.g. Coronaviruses), bacteria (e.g. Campylobacter, Clostridian Summella), and/or multi-cellular organisms (e.g. Teania, Anclostoma, Toxocar., Francopiera, Paragonimus, Strongyloides, Trichuris). Particularly preferred 25 oocysts to be detected include Toxoplasma and Cryptosporidium oocysts. Preferred cysts to be detected include any cysts capable of binding to a solid support and remaining bound to the support through a washing step. Preferred cysts include Giardia cysts. According to this embodiment of the invention, a solid support that is capable of binding cysts or occysts is contacted with a sample of feces, which may or may not have 30 been partially solubilized first in an aqueous solution, and the sample of feces is allowed

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to dry on the support. The solid support can be of any material to which the cysts or oocyts will bind and remain bound during washing in an aqueous solution. The support can comprise one or more compounds that aid in PCR amplification of the sample, for example by allowing the inhibitors to be released in the wash step, or by binding inhibitors of PCR that are not released in the elution step, or by otherwise inactivating inhibitors of PCR amplification. Preferred supports comprise a paper substrate to which the oocysts or cysts can bind. Preferred supports include IsoCodeJ™ Stix, or their equivalent, S&S® #903™, or their equivalent, or Nobuto Blood Filter Strips, or their equivalent. The support, or the portion of the support contacted with the sample of feces, is preferably small enough to fit into a container convenient for the wash step; eg., a size that will fit into a 1.5. ml conical centrifuge tube. The portion of the support that is contacted with the sample of feces can be removed from the rest of the support in order to achieve a convenient size. The portion of the support that includes the dried sample of feces is then washed with an aqueous solution. In a preferred embodiment the aqueous solution is water, preferably distilled water. The solution can comprise one or more compounds that aid in PCR amplification of the sample, for example by inactivating or removing inhibitors of PCR amplification. DNA associated with the sample is eluted by adding an aqueous solution to the support and then heating the solution to a temperature sufficient to elute DNA from the sample, into the solution. In a preferred embodiment, the aqueous solution into which the sample is eluted is water, preferably distilled water. This solution can comprise one or more compounds that aid in PCR amplification of the sample, for example by inactivating inhibitors of PCR amplification, or by improving reaction conditions for the PCR reaction. The heating step comprises heating to a temperature sufficient to elute DNA from the sample. A preferred temperature is approximately 95° C. Oocyst or cyst-specific DNA in the elution solution is then PCR amplified using primers specific to the oocysts or cysts being detected. The amplification products indicative of oocysts or cysts are then detected using any means available for the detection of PCR amplification products. These can include, for example, separation and observation of the PCR products on a

gel, or detection and/or quantification by PCR ELISA. In a preferred embodiment of

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the present invention, nucleic acid molecules of the present invention are used for the detection of *T. gondii* oocysts in cat feces by PCR amplification using nucleic acid molecules of the present invention as primers. According to the present invention, detection of oocysts can be accomplished by direct analysis of feces. Methods to conduct such an assay are described further in the Examples section.

The following examples are provided for the purposes of illustration and are not intended to limit the scope of the present invention.

EXAMPLES

It is to be noted that the examples include a number of molecular biology, microbiology, immunology and biochemistry techniques considered to be familiar to 10 those skilled in the art. Disclosure of such techniques can be found, for example, in Sambrook et al., ibid. and Ausubel, et al., 1993, Current Protocols in Molecular Biology, Greene/Wiley Interscience, New York, NY, and related references. Ausubel, et al, ibid. is incorporated by reference herein in its entirety. DNA sequence analysis and protein translations were carried out using the DNAsis program (available from Hitachi 15 Software, San Bruno, CA) or MacVector program (available from International Biotechnologies, Inc., Hew Haven, CT). It should also be noted that since nucleic acid sequencing technology, and in particular the sequencing of PCR products, is not entirely error-free, that the nucleic acid sequences presented herein represent apparent nucleic 20 acid sequences of the nucleic acid molecules encoding immunogenic T. gondii proteins of the present invention.

Example 1:

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This example discloses the construction of a *T. gondii* genomic expression library.

Pure mRNA from *T. gondii* parasite present in the infected cat gut cannot presently be obtained. Therefore, a true cDNA library for the gametogenic stages cannot be produced. In order to get around the unavailability of pure mRNA from gut stages of *T. gondii*, a genomic expression library in λ gt11 was constructed using Toxoplasma genomic DNA obtained from tachyzoites produced in tissue culture. This library represented genes expressed at all stages of the Toxoplasma life cycle, including the gametogenic genes.

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Construction of the library was modeled on procedures used previously for standard lambda cloning (see, for example, Sambrook, et al., <u>ibid.</u>). In brief, a series of high frequency cutting restriction enzymes were used to generate near random fragments of DNA representing the tachyzoite genome. DNA fragments of approximately 500 to 2000 bp were size selected and then inserted in frame with the expressed fusion protein in λ gt11. Construction of this library is described in greater detail below.

Standard Production of Tachyzoites from liquid nitrogen stocks: Liquid nitrogen stocks of Toxoplasma tachyzoites (TZ) (1 ml samples at 2-4 X 10⁶ TZ/ml) were thawed in a 3^{mC} waterbath. The samples were thawed completely without attaining 37°C. Room temperature TMM (DMEM + 3% FBS + 0.1 ml 50 mg/ml gentamicin per 100 ml media) was added to the thawed sample according to the following timetable: 0.3 ml added at 0 minutes; 0.6 ml added at 5 minutes; 1.5 ml added at 10 minutes. The samples were maintained at room temperature for 5 minutes longer, then centrifuged for 10 minutes at 2.000 RPM at room temperature. The supernatant was discarded and the pellet resuspended in 12 ml of TMM.

Human toreskin fibroblasts (HSF)cells (ATCC CRL 1637) were infected with the thawed tachyzoites as follows: Passage 15-25 HSF cells were split 1:3 and grown to confluence in a 175 flask with DMEM + 10% FBS (fetal bovine serum, available from Summit Biotechnology, Fort Collins, CO) + 0.1 ml gentamicin per 100 ml media in an incubator of 37 (with 5% CO₂. HSF cells were infected by replacing the media with the thawed tachyzoites in TMM. Infections were allowed to progress until 30-50% of the cell monotaver was destroyed. The medium in the infected T75 flask was replaced with tre h 150M the day before harvesting tachyzoites for expansion of the culture.

grown to confluence in a roller bottle incubator apparatus under conditions as described above. The medium from a single roller bottle was decanted and replaced with 100 ml of TMM. The cells in this roller bottle were then infected by adding medium from an infected TTS flask (described above). Infection was allowed to progress until 30-50% of the cell monolayer was destroyed. Fresh TMM was replaced in the infected roller bottle the day before using the supernatant to infect new HSF cells. Four new roller bottles with confluent HSF cells were each infected with 2.5 X 107 tachyzoites harvested from a

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previously infected roller bottle. This cycle of infection of four roller bottles, for the purpose of tachyzoite production, was continued on a weekly basis.

Tachyzoite Purification: Extracellular tachyzoites were collected from tissue culture and concentrated. To collect and concentrate tachyzoites, media from roller bottles containing extracellular tachyzoites were poured into 50 ml conical tubes and centrifuged at 2,000 RPM for 10 minutes. The resulting pellets were pooled and the volume was brought up to 50 ml using TMM. The tachyzoites were diluted and counted using a haemacytometer, and then purified by either the CF-11 column method or the nucleopore method as follows:

CF-11 Method of Purifying Tachyzoites: 1.5g of CF-11 (available from Whatman, Inc., Clifton, NJ) was mixed throughly in 50 ml of DMEM (no FBS), then added to an econo-column chromatography column (available from Biorad, Hercules, CA) and allowed to settle, forming a flat bed. The stopcock was then opened and the excess DMEM was drained until ¼ inch of media remained above the bed. The column was washed by gently adding 50 ml of DMEM and then bringing the media level down to 1 inch above the CF-11 bed. The 50 ml of tachyzoites in TMM (prepared as described above) was then added to the column. The stopcock was opened and the tachyzoites were eluted at a rate of 1 drop/second and collected into 50 ml conical tubes on ice. The media was eluted to ¼ inch above the gel bed. Two additional 5 ml elutions were performed, followed by a 40 ml elution. The 100 ml total eluate was then centrifuged at 2,000 RPM for 10 minutes. The pellets were again pooled by resuspension in 50 ml of DMEM. The tachyzoites were counted and the final number of organisms determined. The tachyzoites were centrifuged at 2,000 rpm for 10 minutes, and the pellet resuspended in 1 ml of Hanks Balanced Salt Solution (HBSS). The tachyzoites were washed 3 times with 1 ml of HBSS by centrifugation at 5000 rpm for 5 minutes in an Eppendorf centrifuge. The pellets were stored at -70°C until needed.

Nucleopore Method of Purifying Tachyzoites: 47 mm nucleopore units (available from Corning Costar Corp., Cambridge, MA) with a polycarbonate 3 um capillary pore membrane were assembled according to manufacturer's specifications. The nucleopore units were then placed on top of an open 50 ml conical tube. Five ml of DMEM was gently forced through the unit using a 30 cc syringe that connects to the top

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of the nucleopore unit. Twenty-five ml of the extracellular tachyzoite preparation collected from tissue culture in DMEM were passed through the unit by gently pushing on the 30 cc syringe. The maximum number of tachyzoites per nucleopore filter did not exceed 5 x 10⁸. Filtration was followed by 2, 5 ml washes of DMEM. The nucleopore-purified tachyzoites were then centrifuged at 2,000 RPM for 10 minutes, and the pelleted tachyzoites resuspended in 50 ml of DMEM. The number of tachyzoites was determined by counting in a hemacytometer. Following centrifugation at 2,000 rpm for 10 minutes, the pellet was resuspended in 1 ml HBSS. The tachyzoites were washed 3 times with 1 ml of HBSS by centrifugation at 5,000 rpm for 5 minutes in an Eppendorf centrifuge. The pellets were stored at -70°C until needed.

Isolation of tachyzoite DNA: DNA from all sources (for example, DNA from Toxoplasma or mammalian tissue) was isolated using standard techniques that can be can be found, for example, in Sambrook et al, *ibid*. In particular, 2X10° tachyzoites were resuspended in 10 ml of 10 mM Tris, pH 8, 0.1 M EDTA, 0.5% SDS and 20 μg/ml pancreatic RNase (available from Sigma Chemical Co., St. Louis, MO). After incubating for 1 hour at 37°C, 1 ml of 5M NaCl and 100 μl of 10 mg/ml proteinase K (available from Boehringer Mannheim Corp., Indianapolis, IN) was added and the solution incubated for 3 hours at 50°C. The solution was then extracted with phenol and the DNA precipitated with EtOH.

Preparation of Restricted and Size Selected DNA: Six, four-base recognition site restriction enzymes, Alu I, Mbo I, Msp I, Rsa I, Sau3A I, and Taqf I, (available from New England Biolabs, Beverly, MA) and one six-nucleotide recognition site restriction enzyme, Dra I, were used to cut T. gondii genomic DNA to completion. Ten µg of tachyzoite DNA was digested to completion according to the manufacturer's recommended protocols for each enzyme. All seven digests of DNA were combined and electrophoresed on an 0.8% preparative agarose gel. The region of the gel representing double stranded DNA between 500 and 2000 bp was excised and the DNA recovered using a Gene Clean Kit (available from BIO 101 Inc., Vista, CA). The eluted DNA was quantitated using an ethidium bromide sensitivity assay on agarose, using calf thymus DNA as a standard. The DNA was then ethanol precipitated.

Addition of Linkers: Four µg of the digested and size selected DNA was then prepared for the addition of linkers by filling in the restriction site overhangs as follows: First, the DNA was resuspended into Klenow buffer, 0.2mM dNTPs, and Klenow fragment (available from Boehringer Mannheim Biochemicals, Indianapolis, IN), and the reaction mix was incubated for 30 minutes at room temperature. The reaction was stopped by incubating the reaction mix at 65°C for 10 minutes. The DNA was then methylated using standard conditions including 0.1 mM s-adenosylmethionine and 120 units of EcoR I methylase (available from Promega Corp., Madison, WI). Following reprecipitation with ethanol, the DNA pellet was dissolved in water and standard T4 DNA ligase buffer (see, for example, Sambrook, et al., ibid.). Three separate EcoR I 10 linkers, constructed to allow three different reading frames (available from Stratagene, La Jolla, CA) were added along with T4 DNA ligase (available from Promega, Corp.) and incubated for 16 hours at 15°C. The solution was then diluted directly into EcoR I restriction buffer and EcoR I enzyme (available from Promega Corp.) and incubated at 37°C for 2 hours. The DNA fragments were separated from the free linkers using a 15 Sephacryl S-400 spin column. The recovered DNA was ethanol precipitated.

Ligation and Packaging of the Restricted DNA: The entire fraction of DNA obtained from the above reaction mixture was ligated into 1 μg of EcoR I-cut and phosphatase treated λ gt11 arms (available from Stratagene) with T4 DNA ligase at 15⁰ C for 16 hours. The phage was then packaged, titered and amplified using the Gigapack[®] II Packaging system (available from Stratagene) according to the manufacturer's directions. The resulting library is referred to herein as the Toxoplasma or T. gondii genomic expression library or as the λ gt11:Toxoplasma genomic expression library.

25 <u>Example 2</u>:

This Example discloses a method of isolation of *T. gondii* nucleic acid molecules encoding immunogenic *T. gondii* proteins recognized by antisera specific for a Toxoplasma intestinal stage: oocysts. This Example further discloses recombinant nucleic acid molecules, proteins and cells of the present invention.

The final stage of *T. gondii* gametogony is the unsporulated oocyst. Antisera was raised directly against Toxoplasma oocysts. In addition to the antisera reacting with their

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respective immunogens, the ability of this antisera to react with *T. gondii* gametogenic stages in intestinal tissue sections from infected animals was assessed. When used in immunofluorescence assays conducted on infected cat gut samples, the anti-oocyst antisera reacted with various parasite structures in the ICG tissue sections, indicating some cross-reactivity with gametogenic stages. This antisera was made as follows.

Production of antibody to a Toxoplasma intestinal stage: oocysts: Oocysts from a wild type strain designated Maggie, a recent isolate from a cat with Toxoplasmosis (Veterinary Teaching Hospital, Colorado State University, 1993), were obtained from the feces of cats fed mouse brains from mice previously infected with the Maggie strain. The oocysts were purified by the standard method of repeated sugar flotation (described in Dubey, J.P. and Beattie, C.P., (1988) *Toxoplasmosis of Animals and Man*, CRC Press, Boca Raton, FL). The oocysts (3 x 10⁷) were vortexed vigorously in 2 ml of PBS, and then frozen and thawed four times using liquid nitrogen and a 37°C water bath. Each thaw was followed with vigorous vortexing. The suspension was then sonicated for 20 seconds. The protein concentration of the sonicate was determined as described above, and the suspension stored at -70° until used.

The thawed oocyst suspension was mixed with Freunds Complete Adjuvant for the first injection and Freund's Incomplete Adjuvant for three subsequent boosts. The protein concentrations of each injection in the series were 9 ug, 50 ug, 90 ug, and 90 ug respectively, delivered at four week intervals. The single cat #1959 (designated Queen 4) used for production of antibody to unsporulated oocysts had been orally infected with 100 mouse brain-derived C strain tissue cysts one month before the initial protein injection. Serum obtained from this cat (designated herein as Q4-1959) was analyzed for the presence of antibody specific to *T. gondii* oocysts by Western blot and immunohistochemistry on a monthly schedule during the injection period.

Immunoscreening the λ gt11:Toxoplasma genomic expression library and isolation of Toxoplasma-specific nucleic acid molecules reactive with antisera to oocysts: Antisera Q4-1959 was used to isolate nucleic acid molecules herein designated OC-1, OC-2, OC-13, OC-14, OC-22, OC-23 as follows: *E. coli* Y1090 was infected with approximately 5X10⁶ plaque forming units (PFU) of the λ gt11:Toxoplasma genomic expression library, and then evenly spread on 20 LB-amp agarose culture

plates. The phage were allowed to grow for about four hours at 37°C. The plates were then overlayed with nitrocellulose filters impregnated with 10 mM isopropyl-B-D-thiogalactoside (IPTG) to induce the expression of the recombinant Toxoplasma protein. The induction proceeded for between 4 hours to overnight and then the filters were marked to establish orientation. The filters were removed and, 5 following several washes in TBST (Tris-buffered saline (TBS) + Tween 20: 20 mM Tris, pH 7.5, 150 mM NaCl, 0.5% Tween-20), and an incubation in blocking solution (TBS + 5% powdered milk), incubated with a 1:40 dilution of antisera Q4-1959 for about 3 hours at room temperature or overnight at 4°C. After 3 to 5 washes with TBST the 10 filters were incubated with a 1:1000 dilution of alkaline phospatase (AP) -conjugated goat anti-cat IgG (available from Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD) at room temperature for two hours. The filters were washed two times with TBST and once with TBS. The color indicator was developed in AP buffer (100 mM Tris pH 9, 100 mM NaCl, 5 mM MgCl) containing 0.7% NBT (nitroblue tetrazolium) and 0.3% 15 BCIP (5-bromo-4-chloro-3-indolyl phosphate).

Plaques in the area corresponding to the positive signals were picked into SM buffer (50 mM Tris, pH 7.5, 100 mM NaCl, 8 mM MgSO₄, and 0.01% gelatin) and the phage replated at a lower density. The same screening procedure was repeated three or four times until a pure plaque was isolated. Of the approximately 5X10⁶ plaques screened in this manner, 6 nucleic acid molecules capable of expressing proteins recognized by antisera Q4-1959 were plaque purified.

Characterization of Immunogenic *T. gondii* proteins encoded by nucleic acid molecules selected from the *T. gondii* genomic expression library:

The nucleic acid molecules identified as positive for expression of immunogenic

T. gondii proteins by immunoscreening with antisera Q4-1959 were screened for expression of proteins reactive with intestinal secretions from immune cats. The production of immune intestinal secretions is described in detail in Example 6, below. Prior to being used for screening, pooled intestinal secretions were preabsorbed with E. coli lysates as follows. Individual cultures of E. coli Y1090 cells and XL-1 blue cells

(available from Stratagene) were grown overnight in LB Amp medium at 37°C. The cells were harvested by centrifugation, then resuspended in PBS, pH 7.4. The cell

suspensions were then frozen and thawed 3 times, using a dry ice-acetone bath and a 37°C water bath, then sonicated on ice for 10 minutes. The protein concentrations of the resulting cell lysates were adjusted to approximately 20 mg/ml, then diluted 1:10 in PBS. Fresh nitrocellulose filters (82 mm) were coated with bacterial proteins by immersing them in the diluted *E. coli* lysates at room temperature for 1 hour. The filters were further incubated in a solution of 4% (w/v) powdered milk in PBS, pH 7.4 for 30 minutes. The filters were then washed with PBS three times for 10 minutes each at room temperature. Pooled immune cat intestinal secretions were diluted 1:20 with 4% (w/v) powdered milk in PBS, pH 7.4. The diluted secretions mixture was incubated with the *E. coli* lysate-treated filters at room temperature for 1 hour, at a ratio of 20 ml per six filters. The resulting absorbed immune intestinal secretions were used without further dilution to screen nucleic acid molecules identified as positive by immunoscreening as described below. Essentially the same protocol was followed when characterizing the proteins expressed by nucleic acid molecules isolated by immunoscreening with other antisera(as described below).

Plaque pure phage identified as positive by immunoscreening were diluted in SM buffer to approximately 50 PFU/3 μ l. 3 μ l of each clone was dropped onto an LB/Amp agar plate which was previously overlayed with top agar containing a 1:20 dilution of a fresh culture of E. coli Y1090 at mid-log growth. The plates were then incubated at 370 C for 5 hours. IPTG-treated nitrocellulose filters were placed on the top agar and incubated for 5 hours. The filters were marked, washed in TBS buffer, pH 8.0 at room temperature for 15 minutes and then blocked with 4% (w/v) powdered milk in TBS for 30 minutes, at room temperature. The filters were incubated with absorbed intestinal secretions at 4°C overnight. All further manipulations were at room temperature. The filters were washed in TBS buffer for 10 minutes, 3 times. The filters were incubated for 2 hours with a 1:300 dilution of horse radish peroxidase (HRP) -conjugated goat anti-cat IgA polyclonal antibody (available from Bethyl Laboratories Inc.) in TBS buffer. The filters were washed in TBS for 10 minutes, 3 times, then incubated with 4-chloro-1naphthol substrate. Clones were judged to be either positive or negative by the intensity of the color reaction relative to wild type phage controls. The results of this assay are summarized in Table 2. Of the six nucleic acid molecules expressing proteins

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recognized by antisera Q4-1959, only OC-1 expressed a protein that was positive for reactivity to immune cat intestinal secretions.

Table 2

Nucleic Acid Molecules Selected with Cat Sera Specific to Unsporulated Oocysts

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CN	ORIGINAL SEO ID NO DESIGNATION		DETECTION	NOIL		EXPRESSION	N C	JAVOR	Ç	VENTOARD	\	
		SOI	nce	7.2	28	pTrCHIS	ACRO	IN VITRO IN VIVO	IN VIVO	SERUM	1 1 1	_
												_
70	OC-1	+	+	+	+	-	ND	ΠN	9	2	+	_
72	OC-2	+	•	2+	+	+	QN	QN	Ð	2		_
74	0C-13	2+	•	+	+	+	ND	+	+	QN		-
92	0C-14	+	•	+	+	•	9	QN	2	2		_
78	0C-22	2+	•	+	2+	+	QN	+	+	9		_
80	0C-23	2+	•	2+	+	+	QN	2	S	2		_
												_

Table 2 Legend:

(ND) indicates not done. In the column labeled "Detection", the numbers associated with the positive responses indicate the intestinal secretion (IS) of the expressed product. In all cases, (+) indicates a positive response, (-) a negative response, and "pDVAC" refers to subcloning into and expression from the eukaryotic expression vector pDVACI, as tested in vitro (BHK infected cat gut cells (ICG), uninfected cat gut cells (UCG), tachyzoites (TZ), and bradyzoites (BZ); "Expression" refers to original name for each nucleic acid molecule; "Detection" represent2s the results of RT-PCR assays to assess cDNA from "SEQ ID NO" is the nucleic acid sequence designation for the nucleic acid molecule; "Original designation" is the results of subcloning the nucleic acid molecule into one or both of two E. coli expression plasmids, pTrCHIS and A CRO; relative signal strength for each primer assayed with each of the four cDNA samples, i.e., ICG, UCG, TZ, and BZ cDNA, cells) and in vivo (mice); "Reactivity", indicates specific recognition by cat immune sera (serum) and/or cat immune and are not a comparison between primers. Some of the nucleic acid molecules identified as positive by immunoscreening were also assessed for expression of proteins reactive with Mozart II (immune) sera. Reactivity was assessed by spotting the purified phage directly on a lawn of host *E. coli* and inducing the expression of protein encoded by the cloned DNA insert using IPTG-soaked filters, similar to the phage screening protocol. The filters were then probed with the Mozart II sera, in essentially the same manner as was used to select the plaque purified phage identified as positive by immunoscreening. The results of these assays are summarized in Table 2.

The Toxoplasma inserts in λ gt11, herein referred to as λ gt11:Toxoplasma nucleic acid molecules were sequenced either by direct sequencing, or by first subcloning the λ gt11:Toxoplasma nucleic acid molecules into a cloning vector, then sequencing. Direct sequencing of each insert was performed as follows: the Toxoplasma-specific insert in λ gt11 was PCR amplified under standard conditions well known in the art using a λ gt11 forward primer (5' GGTGGCGACGACTCCTGGAG 3') and a λ gt11 reverse primer (5' CCAGACCAACTGGTAATGGTAG 3'), and the major PCR reaction product was separated from the rest of the PCR reaction products on a 1% agarose gel. The band representing the major PCR product was excised, and the gel slice was processed using the QIAquick kit (available from Qiagen Inc., Santa Clarita, CA) according to manufacturer's instructions in order to release the DNA. The isolated DNA fragment was sequenced under standard conditions using an ABI PRISM 377 automated DNA sequencer (available from Applied Biosystems, Foster City, CA). Each of the amplification primers were used separately as sequencing primers to obtain sequence from both directions.

Subcloning, then sequencing, was performed as follows: the Toxoplasmaspecific insert was PCR amplified and gel purified as described above. The purified
DNA was then cloned into a TA cloning vector (available from Invitrogen Corp., San
Diego, CA) according to the manufacturer's instructions, and sequenced under standard
conditions.

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Sequence analysis of nucleic acid molecules selected for expression of proteins recognized by antisera Q4-1959:

The nucleic acid molecules selected for expression of proteins recognized by antisera Q4-1959 were sequenced as described above. BLASTn and BLASTp homology searches were performed on these sequences using the NCBI GenBankTM non-redundant (nr) nucleotide (n) and amino acid (p) databases, and the dbEST (est) database as described above. The results of these searches are summarized in Table 3. Nucleic acid molecule OC-1 was sequenced again and some changes found between the first and second sequence. The resequenced nucleic acid molecule is referred to herein as OC-1-a.

rable 3 Homologies

	% sa										1 96	82	8	4 57	29	26		7 85	7 82	88	69	92	8	88	Ţ
	Identities										291301	84102	82102	122214	5176	69122	162237	117137	113137	78/77	5173	2637	2025	121137	
	Clone/Match										302-2/8-308	446-345/8-109	590-489/8-109	349-135/129/342	493-418/129-204	137-16/5-126	363-127/192-428	500-364/340-476	356-220/340-476	601-515/383-469	178-106/350-422	241-205/456-492	373-349/468-492	113-249/340-476	
	Size										553						574			,				574	
HOMOLOGIES	Name							-			1 invivo Bradyzoite cDNA						1 TgME49 invivo Bradyzoite							TgESTzz43d05.s1 TgME49 invivo Bradyzoite	
											TgESTzz29d08.r1						TgESTzz43d05.s1							TgESTzz43d05.s	
TOP HITS	Gene										AA531653						AA520213		i					520213	
T0P	Score										1.20E-112						3.10E-33							4.30E-35	-
-10	p vs nr		•		-		•	٠		•	٠														-
P (N) < 1e-10	n vs est	-	•	•	-	•	•	•	•	•	2													2	
#	n vs nr	•	-	-	•	-	•	•	•	•	•													•	
Size	aa	66	147	142	101	95	230	54	92	183	112														-
S	dq	718	441	428	304	284	069	313	389	248	904	L												549	
SEQ ID		19	21	23	26	28	30	32	34	36	82													88	

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Table 3

Homologies

82 90 75 8 58 8 7 6 96 95 66 97 66 86 96 95 9 82 88 8 89 86 8 198--205 117--0137 147--149 119--137 343--350 211--213 176--185 202--257 245--252 161--236 168--235 93..104 94..122 87..102 77--102 86/102 99--100 93--102 87--102 86--102 54--76 44--46 20.25 1078-1262/206-390 849-1084/220-455 104 530 349 469 498-557/255-336 575-620/334-379 574 114-250/340-476 684-820/340-476 120-195/129-204 237-493/220-476 6 123 458 452 769-804/98-133 329-541/97-309 882-1086/8-212 167-268-8-109 2-105 373-476 671-780/1-100 247-498/2-253 3-151/161-309 452-553/8-109 23 124 8 109 311-4128-109 3-352/127-476 267-501/5-239 411-512/8-109 24-125/8-109 613 ? 380 343 553 574 553 SESTERE OF HIT PARES OF SECTION OF BIRDIES TgESTzz43d05.s1 TgME49 invivo Bradyzoite TgESTzz29d08.r1 TgME49 invivo bradyzoite TgESTzz29d08.r1 TgME49 invivo bradyzoite TgESTzz17b0.r1 TgME49 tachyzoite cDNA TgESTzz43d.s1 TgME49 invivo bradyzoite TgESTzy44c02.r1 TgRH tachyzoite cDNA TgESTzy57e07.r1 TgRH tachyzoite cDNA HOMOLOGIES 4 4 116 . J AA520213 AA012353 AA531653 AA520213 AA531653 N82167 N81503 100 HITS 6.80E-150 1.00E-113 2.40E-142 3.80E-149 8.00E-40 1.20E-33 .50E-37 P (N) < 10.10 ~ N N i 268 289 102 164 227 8 Size 1424 270 306 804 867 680 SEQ ID 87 83 8 6 95

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Table 3 Homologies

			96	71					75	96	9							93	94	83							
			149155	77108					162215	239247	156170							221236	155164	124148							
			3-157/236-390	80-187/283-390					2-216/147-361	161-407/144-390	408-577/390-559							385-150/199-434	642-479/32-195	148-1/435-582							
			553						209									653									
HOMOLOGIES			TgESTzz29d08.r1 TgME49 invivo bradyzoite						TgESTzz69d04.r1 TgME49 invivo bradyzoites									TgESTzz36d07.r1 TgME49 invivo bradyzoite									
TOP HITS			AA531653						AA520348									AA519977			AA520558	AA531849	AA520976	AA274332	W99585	AA520425	AA274257
TOP			4.50E-57						4.50E-176									6.20E-190			9.50E-162	8.90E-122	1.30E-117	4.90E-106	5.20E-102	8.10E-94	8.40E-90
e-10	٠	•			•	•		•				•					•	,									
P (N) < 1e		-	-		•				-			•	•	•	•	,		11									
#						•	•	•						•				·									
Size	66	53	8		139	138		73				8	118	8	129	95	23	34									
"	596	723	270		417	416	200	321	1233			411	441	491	387	310	220	642						L			
SEQ ID	97	66	101		63	65	29	89	54			55	25	29	61	38	40	42									

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Table 3

Homologies

8 86 66 224--224 314--315 316--326 91--92 207-430/91-314 119-418/2-316 119-210/2-93 328-3/11-336 441 577 441 TgESTzz46d07.r1 TgME49 invivo bradyzoites TgESTzz62b09.r1 TgME49 invivo bradyzoite TgESTzz62b09.r1 TgME49 invivo bradyzoite HOMOLOGIES AA532000 AA520558 1.70E-125 AA520558 7.70E-110 AA520339 4.70E-106 AA531849 AA531849 AA520425 8.10E-120 AA532000 8.90E-113 AA520339 AA531849 AA520425 AA012063 AA532000 AA520339 AA520339 AA520558 AA012063 1.70E-87 AA532000 AA012063 AA519977 AA519977 AA274257 AA274257 W99585 W99585 TOP HITS 1.30E-116 4.70E-123 5.50E-116 1.60E-112 4.30E-124 2.20E-110 1.70E-100 1.50E-10 3.20E-95 2.10E-55 1.60E-83 6.80E-59 4.00E-13 7.40E-10 2.40E-97 9.90E-94 8.20E-14 8.20E-53 5.00E-81 P (N) < 1e-10 တ თ ი # 82 32 27 Size 432 282 466 381 SEQ ID 46 48 20 44

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Homologies

						8	96	97								51	8	37			33	8					
						208210	8083	4647								1937	1830	15-40			40118	41123					
						191-400/85-294	108-190/1-83	397-443/290-336								2444 36-72/658-694	42-71/243-272	188-227/893-932			1164 22-139/827-944	6-128/823-945					
						222										2444					1164						
HOMOLOGIES						TgESTzz46d07.r1 TgME49 invivo bradyzoites										Notch protein homolog Homo sapiens					IGA FC/beta antigen Streptococcus agalactiae						
TOP HITS	AA520425	AA519977	AA012063	AA274257	W99585	AA532000			AA531849	AA520339	AA520425	AA519977	AA520558	AA012063	W99585	P46531			A40043	A36666	P27951		FCSOAG	A60234			
TOP	2.60E-97	2.90E-95	1.60E-55	6.40E-14	1.20E-10	9.50E-130 AA532000			9.00E-124	2.50E-109 AA520339	2.90E-98	7.70E-86	8.30E-83	4.40E-55	6.30E-11	2.70E-40			3.60E-40	1.60E-35	1.30E-28		3.40E-28	6.20E-22			
e-10																100					9					-	$ \cdot $
P (N) < 1e						8															•				•	•	•
#																١.					-				•	-	•
Size						82										233					140				101	232	58
S	_	_	_			539										669			_		419				303	969	173
SEQ ID						52										109					111				113	115	117

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Table 3

Homologies

		82	81		8		I		I			I				32								92	8	94	88
		6984	6883		32-92											46140								139142	5356	1819	142144
		1105 459-542/937-1020	542-460/167-249		1749 5-96/1137-1228											10-149/192-331								525-384/56-197	386-331/196-251	542-524/38-56	542-399/136-279
		1105	253		1749								1			378	\downarrow					\downarrow	_	251			401
HOMOLOGIES		T. gondii mitochondria-like REP2	TgESTzy31c05.r1 TgRH tachyzoite	- 1	hypothetical protein: PE Synechocystis.											proline-rich protein Triticum aestivum								TgESTzy39d03.r1			TgESTzy77b07.r1
TOP HITS		X60241	N61888		d1017785	S14959										S14959	d1017785	160171	1372954	S20500	Q15428			N82029			W00112
TOP		2.80E-13	2.90E-13		5.30E-12	7.10E-12										3.60E-15	3.60E-14	1.40E-13	4.10E-13	4.20E-11	2.90E-10			2.00E-72			1.40E-49
6.					2							,	•	٠		9								٠			
P (N) < 1e-			-	,			•			•	•			٠									 -	2	'		
#	ļ .	-	+												,								'				
92	123	1 2	;	205	254		62	190		95	103	178	99	148	19	=						176	125	g	3		
Size	998	Т	Т	616	T		236	269	232	276	309	534	327	444	928	513						528	375	5	3		
SEO ID	119	T		123		T	127	129	131	132	134	136	139	141	143	70						72	74	100	9		

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Homologies

			30	33								ဓ္က	33						86	86					91	5	97
			45149	41122								45149	41122						144146	144146					123134	7272	128131
			1164 22-170/827-975	67-188/824-945								1164 22-170/827-975	67-188/824-945						331-446/4-149	331-446/4-149					369-502/55-188	314-385/1-72	372-502/2-132
Ш			1164									1164							149	149					454		382
HOMOLOGIES			IGA FC/beta antigen Streptococcus agalactiae									IGA FC/beta antigen Streptococcus agalactiae							TgESTzy18d02.r1 TgRH tachyzoite	TgESTzy18d02.r1 TgRH tachyzoite					TgESTzy98f02.r1 TgME49 tachyzoite		TgESTzy55c09.r1 TgRH tachyzoite
TOP HITS			P27951		FCSOAG	A60234	1620100	Q01456	JC4749	d1014692	703450	P27951	FCSOAG	A60234	1620100	Q01456			N61591	N61591					W96667		AA037916
TOP			5.40E-31		1.40E-30	2.60E-22	4.90E-14	1.40E-12	2.50E-10	6.90E-10	8.30E-10	1.70E-27	6.70E-27	1.10E-20	7.80E-14	3.50E-12			1.70E-51	1.70E-51					4.40E-65		3.10E-43
e-10		•	8									5					•		•	-	•	-	-		,		
P (N) < 16	•	•	-									•					-	•	1	1	•	٠		•	2		
#			•									-					,	-	•		٠	•	•	•			
Size	191	612	219									273					142	139	51	62	73	29	119	108	175	Ŀ	
S	573	1835	657									1029					425	417	207	503	322	390	357	339	526		
SEQ ID	78	8	6									11					13	16	17	103	105	107	-	3	5		

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Table 3 Homologies

SEQ ID	S	Size	#	# P (N) < 1e-10	e-10	TOP	TOP HITS	HOMOLOGIES				
_	1478 381	381	·	5		4.60E-128	4.60E-128 W96667	TgESTzy98f02.r1 TgME49 tachyzoite	454	454 864-1126/55-317 251263	251263	95
	_	_								809-868/1-60	7272	100
						4.70E-119	4.70E-119 AA037916					
						4.50E-43 N82635	N82635					
						1.20E-36 N96576	N96576					
						2.20E-36 N82193	N82193					

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Table 3 Legend:

Results of BLASTn and BLASTp search of the NCBI GenBank™ non-redundant (nr) nucleotide (n) and amino acid (p) databases, and the dbEST (est) database. The algorithm used was as described in S.F. Altschul, W. Gish, W. Miller, E.W. Myers, and D.J. Lipman, J. Mol. Biol. 215, 403-10 (1990) and the NCBI. From left to right: are the 5 sequence identification number (SEQ ID No), the size of the nucleic acid molecule (Size) in either base pairs (bp) or amino acids (aa), the number of hits below the sum probability score of 1^{e-10} (# P(N) < 1e-10), and a section of the hits with the highest homology (HOMOLOGIES). The homologies section is sub-divided to include the sum probability (Score) of the homology, the gene accession number (Gene), the name or 10 identifier of the gene (Name), the size of the gene either in nucleotides, if it is a match in the BLASTn or amino acids if it is in the BLASTp (Size), the range of either nucleotides or amino acids in which a match was identified in the clone versus the match in the database (Clone/Match), the number of identities compared with the range matched 15 (Identities), and the percentage homology of the match (%). A dash (-) indicates the search was done and there were no matches.

BNSDOCID: <WO___9932633A1_I_>

WO 99/32633

5

RT-PCR analysis of nucleic acid sequences encoding Immunogenic *T. gondii* proteins:

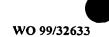
The sequence data obtained as described above were used to design unique primers specific to each nucleic acid molecule of the present invention. These primer sequences are listed in Table 4.

Table 4

Nucleic Acid Molecules Primer Sequences

SEQ ID NO.	ORIGINAL DESIGNATION	NAME	PRIMER SEQUENCE	BASE PAIR
	DEGICIATION			NUMBERS
144		<u> </u>		
145	Tg-41 (5')	nTG1	CGCTTCTTGTGTCACCTG	118
146	Tg-41 (3')	nTG1	GCACCTIGTTCTCTCTCTCGCC	317295
147	Tg-45-2T (5')	nTG2	CGAGGAGACGGTGGGAGC	118
148	Tg-45-2T (3')	nTG2	TGCCCAAGATGCCGATCTCTG	289269
149	Tg-50 (5')	nTG4	TCTCCCCATCGACGAAAAC	95114
150	Tg-50 (3')	nTG4	GCTCATTTCCTCCGCAATTTGG	456435
151	Q2-4 (5')	nTG5	AGCTGGCAGAAATACCAAAGCTC	67-90
152	Q2-4 (3')	nTG5	TGTCGGCAATACTGGGCATG	529-510
153	Q2-9 (5')	nTG6	ACTGGAGTGGAAAGTCTGGTTTTG	3760
154	Q2-9 (3')	nTG6	GACGCAGAGAAGAAGAAGAGCC	415-393
155	Q2-10 (5')	nTG7	TCCAAAACTGTCTCGTCTCCCC	165186
156	Q2-10 (3')	nTG7	TCTGGATACGCCGTTCCTTTG	305284
157	Q2-11 (5')	nTG8	GACATCTACCTGTGAGTGAACCAGG	5074
158	Q2-11 (3')	nTG8	GTCAAAACCTTGCCAGCATCTC	475454
159	4499-9 (5')	nTG9	TCCGACTGAATGACTACCTCTTTC	4528
160	4499-9 (3')	nTG9	TCCGACCAAGTCCTCAGTGAAC	537516
161	4604-2 (5')	nTG10	TGGGCATTTCCTGGAAGAGG	3655
162	4604-2 (3')	nTG10	GAATCCATCTCGTGCAAACGG	378358
163	4604-3 (5')	nTG11	CAAGACACAGGGAAACGTTGG	102122
164	4604-3 (3')	nTG11	GAAAGAATCGCACCTCCTCTCC	424-403
165	4604-5 (5')	nTG13	TTTGAGTCTAACCGCCGTATGTC	20-42
166	4604-5 (3')	nTG13		216194
167	4604-10 (5')	nTG15	TCGACTTGGGTCCGATTGTTAG	4364
168	4604-10 (3')	nTG15	GATCTTTTGCGTGACTTTGTCTGC	289266
169	4604-17 (5')	nTG16		1942
170	4604-17 (3')	nTG16	GAGGGTTTCCTTCTTTATTGCC	178156
171	4604-54 (5')	nTG17	TGTTGGACATCCCGAGCATC	23-42
172		nTG17	GGTCCTTGTTTTTCAGGCGG	472-453
173		nTG18	TCGTGCAGACAGTGAAGCAATG	3556
174		nTG18	TTTTGTCAGCACAGAGTGGCG	201–281
175		nTG19	CGCAAGTGAGTTTTGGCTTTACC	1537
176			CCTGGAAGAGATATGCAGACAC	389368
177			TCACCGTTCGCTCTTCTTCTC	1233
178			CGACTGAAGCATGGATTGCC	367348
			ACATATTCCTGAGGAGGAGTTCCC	82-105
180			AACACACCTCCGACGACACCAC	447426
			CTCGGCTTCTCCACATACAAGG	829
			GGATCTAGGCATTTGGGTTTCAC	411389
			ATCGAAGAAGCTGAAGCGGAG	424
			GTGCTTGTCTCTGACGAAACCC	193172
			TATCATTGTATCCCGTCGTCCC	4768
			TGATGCCTGGATTTGCACAAC	363-343
187	AMX/I-10 (5')	nTG35	CGGATCGCTCTGAGTCTCTTTG	122

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Table 4

Nucleic Acid Molecules Primer Sequences

188	AMX/I-10 (3')	nTG35	ATCCTGTGTCTTCTCTTCGACCC	384362
189	AMI-23 (5')	nTG36		88110
190	AMI-24 (5')	nTG37		21-44
191	AMI-24 (3')	nTG37		347–326
192	AMI-28 (5')	nTG38		21-43
193	AMI-28 (3')	nTG38		440-419
194	AMI-47 (5')	nTG40		95-115
195	AMI-47 (3')	nTG40		252230
196	OC-1 (5')	nTG41	CCCGAAGACTTTGACCTG	3451
197	OC-1 (3')	nTG41	AGTGGCATAGGAGGCTGG	191174
198	OC-2 (5')	nTG42	GCACCTTCAATGCCACAGGTATC	90-112
199	OC-2 (3')	nTG42	TCGTGTGCTTCTCGCTTCTCTG	484-463
200	0C-13 (5')	nTG43		84108
201	0C-13 (3')	nTG43	GCTCCGTGGGCACATTTTTG	367348
202	0C-14 (5')	nTG44		933
203	0C-14 (3')	nTG44	GATTGCGTGGGCAGTGTAGAAG	237-216
204	0C-22 (5')	nTG45	TGTTTGTTTCCCCAGTCAACGAC	89111
205	0C-22 (3')	nTG45	CGGAAGAGGTTGTTGGACTCCTTC	570547
206	0C-23 (5')	nTG46	CAACCGAGAGAGAGAGAGAACAG	62-86
207	0C-23 (3')	nTG46	TGGGGAGAACAGCAGACATCAG	602-581
208	4CQA11 (5')	nTG49	GGATGAACACTGGTGCATCATG	627
209	4CQA11 (3')	nTG49	CGACTTGGTCCGCTC	270256
210	4CQA19 (5')	nTG50	CGGCGCAACAATGGGC	1-18
211	4CQA19 (3')	nTG50	GTCCGAGATATGAGGATGCGAC	129108
212	4CQA21 (5')	nTG51	TCAGAGCACCATTGTTGCGAC	39-59
213	4CQA21 (3')	nTG51	TTTGACGCTCAAGTGGAGGCTG	556535
214	4CQA22 (5')	nTG52	GCCTGCAACGCTCGATGGC	615-633
215	4CQA22 (3')	nTG52	CTTCTTGACTACCTTCACGTCTG	810788
216	4CQA24 (5')	nTG53	AAGGACAAGCCTGGTTTG	283-300
217	4CQA24 (3')		TTTGCCCTTCGCACAATC	11301113
218	4CQA25 (5')		CCAGTTTTGCCAGAGGAAGACC	82-103
219	4CQA25 (3')		ATCCGTCAATGCAGGTTTCATC	459-438
220	4CQA26 (5')		AGACACCAGAGACAGCAGCAGTC	4567
221	4CQA26 (3')		ACTTCGCCCGACAATCGCTTTCC	266244
222	4CQA27 (5')	nTG56	CGATCCTCCCGAGGGACC	118
223	4CQA27 (3')		GCCTTTACGCATTCAAGTCGTG	174–153
224	4CQA29 (3')	nTG57	TTCAGCGGGTCTTTCCTCAC	129110
225	R8050-2 (5')		CAACGAGAAAGATGGAGCTTCG	3455
226	R8050-2 (3')		AACTTCTTGCACTTGGTCCCG	404384
227	R8050-5 (5')	nTG60	AAGCGAGGAAAAGGAGGTGTCTC	95-118
228	R8050-5 (3')	nTG60	004400	250-230
229	R8050-6 (5')	nTG61	T000001001	8-3O
230	R8050-6 (3')	nTG61		254–230
231	M2A1 (5')	nTG62	007000	223
232	M2A1 (3')			341–320

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Table 4

Nucleic Acid Molecules Primer Sequences

233	M2A3 (5')	nTG64	TTGTTCTCGAACCCGCAGAG	74-93
234	M2A3 (3')	nTG64	TGGCAAGAGACCGAATCGTG	235216
235	M2A4 (5')	nTG65	AAACTTGGCAAAGGGGAACG	4968
236	M2A4 (3')	nTG65	TGCTGTGGAGAATGATGGCTG	483463
237	M2A5 (5')	nTG66	TTTCCGACGAAGCTGCC	25-41
238	M2A5 (3')	nTG66	GACTCCAACGAAAGCCTCG	144126
239	M2A6 (5')	nTG67	GGAAAGGGATAAAGACGCCG	150169
240	M2A6 (3')	nTG67		337-314
241	M2A7 (5')	nTG68	CTGCACCATTTCTCACTTCTTGTG	5780
242	M2A7 (3')	nTG68	GCAAAAGCGGACTCGATTCTATTG	192169
243	M2A11 (5')	nTG69	TGTGGCAGAGCAAAAGGCTC	1231
244	M2A11 (3')	nTG69	CTGTGGATGCTCCTTTGCGACT	406385
245	M2A16 (5')	nTG70	CGAGGCACCCGAAGAATTTG	195214
246	M2A16 (3')	nTG70	CTTCTCAGGTTCACTTCCTGCG	759738
247	M2A18 (5')	nTG71	TCACGCAACGAACAAGTCCTC	4262
248	M2A18 (31)	nTG71	CCCATTTTTGCTTGGCTTGC	149-130
249	M2A19 (5')	nTG72	AGCGGCAAACCAGTTCGTTG	283-302
250	M2A19 (3')	nTG72	CACCACCTTTTTCGTTGCGG	558539
251	IM2A20 (5')	nTG73	CGGCGACTCAGATGGG	116
252	M2A20 (3')	nTG73	GGGGCTGTGTCTTCTCTATTTCG	131109
253	M2A21 (5)	nTG74	AAGCAAACAGGCTCGGAAGC	127146
254	M2A21 (3)	nTG74	TCATGTTGGAGGCGTCGTTC	241222
255	M2A22 (5)	nTG75	TGTGCAGTGGAGGACAAATGG	5070
256	M2A22 (3)	nTG75	GAATCAGGGTGTTTTAGGGCG	284264
257	[M2A23 (5)		ATTCTGTGCAAGCCCAGAG	305-323
258	M2A23 (31)	nTG76	CGACCAAGGGTGTTGACCAT	136155
259	MCA24 (5')	nTG77	CTAGGCAAAGAAACACCCATGC	226-247
260	M2A24 (31)	nTG77	CGCTGGAACTCCTGACAC	327310
261	M2A25 (5)	nTG78	ACGAAGGGAGAGATGCGTTTG	5979
262	M2A25 (3)	nTG78	TGGCTGTTTGGGTTGTCTGG	392-373
263	M2A29 (5)	nTG79	TCACCGCAGAACTTAACCCG	6281
264	M2A39 (3)	nTG79	CTCGCTTTTCCAGCTTGTCG	249230

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PCT/US98/27137

Table 4 Legend:

5

Primer Sequences to Nucleic Acid Molecules. The original name (Original Designation) and the present name (Name) for each nucleic acid molecule are listed in the second and third columns. Separate 5' and 3' primer sequences are listed for the nucleic acid molecules under Primer Sequence. Identification of each primer sequence as 5' or 3' is shown in the column labeled Original Designation. The location of each primer sequences in its respective nucleic acid molecule is shown in the column, Base Pair Numbers. The sequence identification number for each primer is listed in the first column (Seq ID NO).

BNSDOCID: <WO___9932633A1_I_>

The unique primers listed in Table 4 were used in reverse transcriptase-polymerase chain reaction (RT-PCR) assays to assess the expression of the particular nucleic acid sequence in ICG, bradyzoites and tachyzoites. DNA templates were generated from total or poly A+ RNA using an RT-PCT kit (available from Stratagene) according to the manufacturer's instructions. The resulting DNA templates were then amplified by standard PCR reaction. The RT-PCR reactions were performed using RNA isolated from infected cat gut (ICG), bradyzoites (BZ), tachyzoites (TZ), and the appropriate controls (e.g., uninfected cat gut (UCG) RNA). In addition to UCG controls, clone-specific primers were used in PCR reactions using DNA from the following sources: *T. gondii*, mouse cells, cat intestinal cells, and human cells. These results are summarized in Table 2.

Subcloning *T. gondii* nucleic acid molecules encoding Immunogenic *T. gondii* proteins into the expression vector pTrcHisB:

T. gondii nucleic acid molecules encoding immunogenic T. gondii proteins isolated as described above were subcloned into the expression vector pTrcHisB (available from Invitrogen Corp., San Diego, CA). The vector pTrcHisB is designed for expression of fusion proteins in E. coli and purification of proteins encoded by nucleic acid molecules of interest. Expression of fusion proteins from this vector was assessed following induction and subsequent Western blot analysis of the E. coli lysates using both a monoclonal antibody to the T7 phage amino acid tag sequence and the original sera used to select the nucleic acid molecule. The fusion proteins all contain a poly histidine amino acid sequence which was used to purify the fusion proteins using metal chelate chromatography.

Recombinant molecules containing nucleic acid sequences encoding

25 immunogenic *T. gondii* proteins were produced by PCR amplifying plaque purified λ gtl1:Toxoplasma nucleic acid molecules using a λ gtl1 forward primer (5' GGTGGCGACGACTCCTGGAG 3') and a λ gtl1 reverse primer (5' CCAGACCAACTGGTAATGGTAG 3'). Amplifying the Toxoplasma inserts in this way produced DNA fragments with *Eco*R I sites at the junctions between the

30 Toxoplasma insert and the lambda vector. These PCR fragments were then digested with the restriction endonuclease *Eco*R I, gel purified and subcloned into the *Eco*R I-

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cleaved expression vector, pTrcHisB. The resultant recombinant molecules were transformed into DH5a competent cells to form recombinant cells, and assayed for the expression of an immunogenic *T. gondii* protein. The results of these assays are summarized in Table 2.

The recombinant cells were cultured in enriched bacterial growth medium containing 0.1 mg/ml ampicillin and 0.1% glucose at about 37° C. When the cells reached an OD_x, of about 0.4-0.5, expression of recombinant proteins was induced by the addition of 0.5 mM isopropyl-B-D-thiogalactoside (IPTG), and the cells were cultured for about 4 hours at about 37° C. Immunoblot analysis of the recombinant cell lysates using a 17 tag monoclonal antibody (available from Novagen Inc., Madison, WI) directed against the fusion portion of the recombinant Toxoplasma fusion protein was used to confirm the expression of the fusion proteins and to identify their size. In addition, the original selecting antisera were used to determine whether the recombinant expression neclecule expressed a protein that could be recognized by the sera originally used to replace the Toxoplasma-specific portion of the recombinant molecule. The results of these immunoblot assays are summarized in Table 2. Of the six nucleic acid molecules selected by immunoscreening with antiserum raised against oocysts (Q4-1959 serum). Six were positive by this immunoblot assays.

Examele 3

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20 This I sample discloses a method of isolation of *T. gondii* nucleic acid molecules encoding immunogenic *T. gondii* proteins recognized by antisera raised against the initiating stage of *T. gondii* gametogony: the bradyzoite. This Example further discloses recombinate to a less acid molecules, proteins and cells of the present invention.

brain tissue extra were used to generate stage-specific antibody to *T. gondii* as follows:

harvesting to one cysts from chronically infected mice that had been infected, either intraperatoneally with tachyzoites produced *in vitro*, or by oral gavage with tissues cysts. Between tour and eight weeks post-infection, tissue cysts were harvested and used to inoculate naive mice. Harvest was accomplished by dissecting out the brains of infected mice cuthanized by inhalation of CO₂. The brains were added to a tube of 30% Dextran

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in HBSS (Hanks Balanced Salt Solution, available from Life Technologies Inc. (Gibco/BRL), Gaithersburg, MD), and placed on ice until further purified. Each tube contained a maximum of 8 brains per 20 ml of 30% Dextran solution. Tissue cysts were purified by homogenizing the brains for 20-30 seconds with a Tissuemizer (available from Tekmar-Dohrmann, Cincinnati, OH). The homogenized brains were centrifuged for 10 minutes at 3,300 g at 4° C. The supernatant was poured off and the pellet was resuspended in 2.0 ml of HBSS. The pellets from multiple tubes were combined and the tissue cysts were counted using a hemacytometer. To produce a new lot of chronically infected mice, tissue cysts purified as described above were diluted in HBSS to a concentration of 100 tissue cysts/ml. Mice were inoculated by oral gavage with 100 µl (10 tissue cysts). After six weeks there were approximately 600 tissue cysts per mouse.

Bradyzoites were purified from tissue cysts by pepsin digestion and passage through a CF-11 cellulose column. Pepsin digestion was initiated by adding approximately 1.0 ml of pepsin digestion fluid (0.5% pepsin, 0.17 M NaCl, and 1.16 M HCl) fluid per 1.0 ml of cyst suspension. The sample was incubated for 10 min in a 37°C waterbath with occasional swirling. After incubation, approximately 0.9 ml of 0.5% sodium carbonate per 1.0 ml of sample was added slowly and with constant gentle mixing. The solution was then centrifuged for 10 minutes at 2,000 rpm. The supernatant was removed and the pellet resuspended in 5.0 ml of Dulbecco's Modified Eagle's Medium.

1.2 g of CF-11 cellulose was added to 50.0 ml of DMEM, and then poured into a 50 ml chromatography column. The column was equilibrated by allowing most of the DMEM to wash out. The pepsin-digested bradyzoites were diluted with 45 ml of DMEM and loaded onto the column. The column was allowed to drip slowly and the flow through was collected. The column was washed with another 50 ml of DMEM and the flow through was again collected. The two 50 ml flow through aliquots were centrifuged at 2,000 rpm for 15 min. The supernatant was carefully removed and the bradyzoite pellet was resuspended in 1ml of sterile PBS buffer. The number of bradyzoites obtained was determined by counting an aliquot using a hemacytometer.

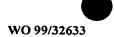
Bradyzoites prepared as described above were lysed in a PBS, 0.001% Triton X-100 solution by freeze-thawing four times in liquid nitrogen and a 37°C water bath. The

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resulting lysate was further treated by sonication for ten, 30 second bursts, while on ice. Following protein determination using a BCA Protein Kit (available from Pierce Biochemicals, Rockford, IL), the bradyzoite lysate was mixed with Freunds Complete and Freunds Incomplete Adjuvants for the first and subsequent (booster) injections respectively. The first injection of rabbit #2448 contained 46 mg of soluble protein, and the two following boosts contained 6 ug of soluble protein each. Injections were given subcutaneously at four week intervals, and serum, designated 2448, was collected every three weeks.

Antiserum 2448 was used to isolate nucleic acid molecules herein designated BZ1-2, BZ1-3, BZ1-6, BZ2-3, BZ2-5, BZ3-2, BZ4-3 and BZ4-6 as follows: *E. coli* Y1090 was infected with approximately 2X10⁵ PFU and then evenly spread on 4 LB-amp agarose culture plates. The rest of the screening procedure was as described for immunoscreening with antisera Q4-1959 (Example 2), with the following exceptions: the primary antibody was used at a 1:200 dilution, and the secondary antibody was a 1:1000 dilution of AP-conjugated goat anti-rabbit IgG. Of the 2X10⁵ plaques screened in this manner, 8 nucleic acid molecules capable of expressing proteins recognized by antisera 2448 were plaque purified.

<u>Characterization of Immunogenic T. gondii</u> proteins encoded by nucleic acid molecules selected from the T. gondii genomic expression library:

The nucleic acid molecules identified as positive for expression of Toxoplasma stage-specific antigenic proteins by immunoscreening with antisera 2448 were screened for expression of proteins reactive with intestinal secretions from immune cats, as described above. The results of this assay are summarized in Table 5. None of the 8 nucleic acid molecules expressing proteins recognized by antisera 2448 were positive for reactivity to immune cat intestinal secretions in this assay.

Table 5

Nucleic Acid Molecules Selected with Rabbit Sera Specific to Bradyzoites

S REACTIVITY SERUM 99999999 IN VITRO IN VIVO 99999999 **pDVAC** 99999999 ACRO 2222222 **EXPRESSION** pTrCHIS 222222 9999 9999 82 2222222 DETECTION nce 9999999 <u>9</u> 99999999 DESIGNATION ORIGINAL BZ1-3 BZ1-6 BZ2-3 BZ2-5 BZ3-2 BZ4-3 BZ4-6 SEQ ID NO 2 2 2 38 9 44 46

Table 5 Legend: See Legend for Table 2.

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Sequence analysis of nucleic acid molecules selected for expression of proteins recognized by antisera 2448:

The nucleic acid molecules selected for expression of proteins recognized by antisera 2448 were sequenced as described above. BLASTn and BLASTp homology searches were performed on these sequences using the NCBI GenBankTM non-redundant (nr) nucleotide (n) and amino acid (p) databases, and the dbEST (est) database as described above. The results of these searches are summarized in Table 3. Nucleic acid molecule BZ1-2 was sequenced again and some changes found between the first and second sequence. The resequenced nucleic acid molecule is referred to herein as BZ2-1-a.

Example 4:

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This Example discloses a method of isolation of *T. gondii* nucleic acid molecules encoding immunogenic *T. gondii* proteins recognized by rabbit antisera raised against infected cat gut. This Example further discloses recombinant nucleic acid molecules, proteins and cells of the present invention.

Production of rabbit antisera to infected cat gut: A pregnant female cat (designated Queen 2) (available from Liberty Laboratories, Liberty Corners, NJ) was maintained in isolation and allowed to come to term. The kittens (4) were housed with the mother and nursed normally throughout the protocol. At day seven post-partum, one kitten was selected as the control and its intestine harvested as described below. The remaining kittens were infected orally with 5000 mouse brain-derived tissue cysts of the *T. gondii* strain C, by dripping a solution of the tissue cysts in 1 ml of PBS down the back of their throats. The infected kitten intestines were obtained and processed on day 7 post-infection. The Queen 2 was also infected orally at the same time and in a similar fas2hion using 100 tissue cysts of *T. gondii* C strain.

In order to obtain fresh intestine, the following procedure was used for both the control and infected animals. A kitten was first anesthetized by placing it in an inhalation chamber which was flooded with both isoflurane and oxygen until the animal was anesthetized. The kitten was then euthanized with an intravenous injection of commercial pentobarbital euthanasia solution at the recommended dose (88 mg/kg). The animal was immediately dissected to expose the small intestine. This was removed by

excisions at the anterior junction with the stomach and the posterior junction with the large intestine. The intestine was opened by a single cut from the anterior to the posterior end, exposing the mucosal surface. The gut was then dipped sequentially into three separate washing baths containing cold HBSS (Hanks buffered saline solution) (available from Life Technologies Inc. (Gibco/BRL), Gaithersburg, MD). The intestine was then placed flat on a chilled laminated sterile surface with the mucosal layer up. A single piece of dry nitrocellulose (BA85, available from Schleicher and Schuell Inc., Keene, NH) the length of the intestine, ranging in size from 40 to 70 cm long (this varied with the animal) and 5mm wide, was carefully placed lengthwise on the mucosal surface of the intestine to obtain an impression smear of the villus epithelial cells. After the nitrocellulose strip became wet (approximately 30 seconds after application), the strip was carefully lifted off and allowed to air dry. The orientation of the anterior and posterior ends of the intestine and strip were noted. Forty biopsy samples, approximately 4 mm by 4 mm sections, were then taken from random positions throughout the length of the intestine, and immediately fixed in either methanol or gluteraldehyde, and maintained for further histological analysis. The intestine was then cut into ten equal sections, and each section placed in a separate bag, labeled and quick frozen in a dry ice and acetone bath. The intestinal sections were maintained at -70° until further processing.

Sections of the cat gut which contained *T. gondii* were identified using PCR analysis of the DNA captured by the nitrocellulose lift with primers specific to the *T. gondii* α-tubulin gene. The presence of *T. gondii* parasite infection was confirmed by histological analysis of the biopsy sections. Portions of *T. gondii*-positive cat gut sections were then prepared as follows for subsequent injections into rabbits to produce antibody directed toward major epitopes from *T. gondii* gametogenic stages. The same methods were also used to produce antibody in cats to infected cat gut preparations, as herein described (in Example 5). A piece of intestine approximately 2 mm by 20 mm was cut from five frozen sections of infected cat gut material. The pieces were maintained at 4°C, laid flat and the mucosal layer carefully scraped from the intestine wall and muscle layers using a razor blade. This material was then minced and placed in 5 ml of sterile PBS containing 1% nystatin, 10 μg/ml gentamicin, and 1%

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penicillin/streptomycin in a conical centrifuge tube. The mixture was brought through 4 cycles of a freeze-thaw treatment using liquid nitrogen and a 37°C waterbath. The sample was vortexed between each cycle. The sample was placed on ice and then sonicated using a microtip for 20 seconds followed by 20 seconds on ice. This was repeated four times. The suspension was divided among four Eppendorf tubes and centrifuged (Eppendorf 5415C centrifuge, available from Brinkmann Instruments Inc., Westbury, NY) at maximum speed for 30 minutes at 4°C. The supernatant was then put through a 0.22 micron filter and a protein determination performed using the BCA Protein Determination Kit (available from Pierce Chemical Co., Rockford, IL) according to the manufacturer's protocol. The sample was stored as small aliquots at -70°C until used.

Polyclonal antisera against infected cat gut (ICG) antigens (also herein referred to as anti-ICG antiserum, or anti-ICG antibody) were prepared by immunization of New Zealand White rabbits with infected cat gut tissue protein as follows. Six rabbits were injected with the solubilized cat gut material; two rabbits (designated #4603 and #8049) were injected with solubilized material from uninfected cat gut, and 4 rabbits (designated #4604, #4499, #8050, and #8051) were injected with solubilized material from infected cat gut material. For the first injection, 0.5 mg of soluble protein, prepared as described above, was brought to 0.5 ml and mixed with an equal volume of Freunds Complete Adjuvant. This solution was delivered sub-cutaneously (SQ). The second injection, two weeks later, was identical to the first, except Freunds Incomplete Adjuvant was used. A third injection, twelve weeks after the first injection, was similar to prior injections except that the total amount of protein injected was 1.5 mg. The animals were pre-bled prior to the first immunization and were bled at approximately monthly intervals to monitor antibody responses. The blood was allowed to clot at room temperature and serum obtained by centrifugation. The sera were evaluated for the presence of antibody specific to T. gondii by both Western blot analysis using tachyzoite lysates and by indirect immunofluorescent antibody assay (section IFA) using histological sections obtained from infected cat intestine.

The rabbit antisera were preabsorbed to uninfected cat gut material prior to use in immunoscreening, either by absorbing the antisera to Sepharose beads to which

solubilized uninfected cat gut material had been covalently linked, or by absorbing the antisera to nitrocellulose sheets to which uninfected cat gut protein was bound. Western analysis demonstrated that greater than 98% of the serum reactivity to uninfected cat gut was removed by preabsorption to the column. The remaining (unabsorbed) sera showed reactivity towards *T. gondii* tachyzoite lysates. The unabsorbed sera were used to screen the Toxoplasma genomic library.

Antisera 4604 was used to isolate nucleic acid molecules herein designated 4604-2, 4604-3, 4604-5, 4604-10, 4604-17, 4604-54, 4604-62, 4604-63 and 4604-69 as follows: Two separate immunoscreens were performed with this antisera, and

Toxoplasma-specific nucleic acid molecules were isolated form each screen. In the first screen, *E. coli* Y1090 was infected with approximately 5X10⁴ PFU and then evenly spread on 10 LB-amp agarose culture plates. In the second screen, *E. coli* Y1090 was infected with approximately 1.5X10⁶ PFU and then evenly spread on 12 LB-amp agarose culture plates. The rest of the screening procedure was as described for

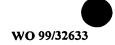
immunoscreening with antisera Q4-1959 (Example 2), with the following exceptions: the primary antibody was used at a 1:500 dilution, and the secondary antibody was a 1:500 dilution of AP-conjugated goat anti-rabbit IgG. Of the approximately 1.5X10⁶ plaques screened in this manner, 15 nucleic acid molecules capable of expressing proteins recognized by antisera 4604 were plaque purified.

Antisera 4499 was used to isolate nucleic acid molecule 4499-9 as follows: *E. coli* Y1090 was infected with approximately 5X10⁴ PFU and then evenly spread on 10 LB-amp agarose culture plates. The rest of the screening procedure was as described for immunoscreening with antisera Q4-1959 (Example 2), with the following exceptions: the primary antibody was used at a 1:200 dilution, and the secondary antibody was a 1:500 dilution of AP-conjugated goat anti-rabbit IgG. Of the 5X10⁴ plaques screened in this manner, 2 nucleic acid molecules capable of expressing proteins recognized by antisera 4499 were plaque purified.

Antisera R8050 (rabbit antisera raised against infected cat gut) was used to isolate nucleic acid molecules herein designated R8050-2, R8050-5, and R8050-6 as follows: *E. coli* Y1090 was infected with approximately 5X10⁶ PFU and then evenly spread on 10 LB-amp agarose culture plates. The rest of the screening procedure was as

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described for immunoscreening with antisera Q4-1959 (Example 2), with the following exceptions: the primary antibody was used at a 1:200 dilution, and the secondary antibody was a 1:1000 dilution of AP-conjugated goat anti-rabbit IgG (available from Kirkegaard Perry Laboratories). Of the 5X10⁶ plaques screened in this manner, 4 nucleic acid molecules capable of capressing proteins recognized by antisera R8050 were plaque purified.

Selected nucleic acid molecules identified by screening for the expression of proteins recognized by rabbit anti-ICG antisera were subcloned and sequenced as described in Example 2. The results of assays to characterize the isolated nucleic acid molecules are summarized in Table 6.

Table 6

Nucleic Acid Molecules Selected with Rabbit Sera Specific to Infected Cat Gut

	Т	Г		Γ		Г	Г	1			
REACTIVITY	SI	+				-	,		,		1
REAC	SERUM	•	Q	Q	9	S	QN	Q	Q	2	Q
DVAC	IN VIVO	L1D	QN	Q	QN	2	ND	DN	QN	QN	QN
Q	IN VITRO IN VIVO	110	QN	QN	QN	ND	DN	QN	QN	QN	QN
SION	CRO	•	•	+	•	•	•	+	QN	QN	+
EXPRESSION	PTICHIS	ND	ND	QN	QN	ND	ND	Q	+	.,	2
	87	•	٠	•	+	+	+	2+	+	+	+
CTION	12	•	+	+	+	+	+	-	+	+	2+
DETECTION	000		٠	•	•	,	•	•	•		•
	ပ္	•	•	+	,	•	+	+	+	+	+
ORIGINAL SEQ ID NO DESIGNATION		0 50.77	4604.2	4604-3	4604-5	4604-10	4604-17	4604-54	4604-62	4604-63	4604-69
SEQ ID NO		19	21	23	25	26	28	30	32	34	36

2	ND	S	2	•	•	2+	1	+	R8050-6	107
2	ND	QN	2	+	-	+	•	,	R8050-5	105
~	ND	Q	Q	+	+	2+	•	+	R8050-2	103

Table 6 Legend: See Legend for Table 2.

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Sequence analysis of nucleic acid molecules selected for expression of proteins recognized by rabbit anti-ICG antisera 4604, 4499 and R8050:

Nucleic acid molecules 4604-2, 4604-3, 4604-5, 4604-10, 4604-17, 4604-54, 4604-62, 4604-6, 4604-69, R8050-2, R8050-5, and R8050-6 were sequenced as described above. These nucleic acid molecules were sequenced as described above. BLASTn and BLASTp homology searches were performed on these sequences using the NCBI GenBankTM non-redundant (nr) nucleotide (n) and amino acid (p) databases, and the dbEST (est) database as described above. The results of these searches are summarized in Table 3., as described above. The results of these searches are summarized in Table 3.

The sequence data described above were used to design unique primers specific to each nucleic acid molecule of the present invention. These primer sequences are listed in Table 4. The unique primers listed in Table 4 were used in reverse transcriptase-polymerase chain reaction (RT-PCR) assays to assess the expression of the particular nucleic acid sequence in ICG, bradyzoites and tachyzoites. The results of these assays are summarized in Table 6.

T. gondii nucleic acid molecules encoding immunogenic T. gondii proteins isolated by immunoscreening with rabbit anti-ICG antiserum were subcloned into either or both of two expression vectors: pTrcHisB (as described above) or

20 Prcro/T2ori/RSET-B (described below). Expression of the fusion proteins from these vectors, and purification of their expressed fusion proteins, were as described above. The results of assays for the expression of recombinant immunogenic *T. gondii* proteins from these expression vectors is summarized in Table 6.

Recombinant nucleic acid molecules and protein molecules including sequences
25 encoding *T. gondii* antigenic proteins and sequences from the vector Prcro/T2ori/RSET
<u>B</u>: Recombinant molecules containing *T. gondii* nucleic acid molecules operatively linked to lambda phage transcriptional control sequences and to a fusion sequence encoding a poly-histidine segment, were produced in the following manner. *T. gondii*DNA fragments in λ gt11 were PCR amplified from nucleic acid molecules herein designated 4499-9, 4604-2, 4604-3, 4604-5, 4604-10, 4604-17, 4604-54, and 4604-69, using the λ gt11 forward and reverse primers herein described. Recombinant molecules

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were produced by digesting the PCR product with EcoR I, gel purifying the resulting fragment, and subcloning into expression vector PRcro/T2ori/RSET-B (also referred to herein as λ CRO) that had been cleaved with EcoR I and gel purified. Expression vector PRcro/T2ori/RSET-B contains the following nucleotide segments: An about 1990-bp Pvu II to Aat II fragment from pUC19 containing the ampicillin resistance gene and E. coli of replication; an about 1000-bp Pvu II to Bgl II fragment from pRK248cIts (available from American Type Culture Collection, Rockville, MD) containing lambda transcriptional regulatory regions (including the gene encoding cI^{ts} , the promoter p_R , and a sequence encoding 22 amino acids of the cro protein); an about 60-bp BgI II to Xba I fragment from pGEMEX-1 (available from Promega Corp.) which contains the T7 10 promoter; an about 166-bp Xba I to EcoR I fragment from pRSET-B (available from Invitrogen, San Diego CA) which contains sequences encoding the T7-S10 translational enhancer, the His, fusion, the 14-amino acid S10 leader fusion, and an enterokinase cleavage site as well as the multiple cloning site; and an about 210-bp EcoR I to Aat II 15 fragment containing synthetic translational and transcription termination signals including the T₁ translation terminators in all three reading frames, an RNA stabilization sequence from Bacillus thurengiensis crystal protein and the T2 rho-independent transcription terminator from the trpA operon. Expression vector PRcro/T2ori/RSET-B contains the following nucleotide segments. An about 1990-bp PvuII to AatII fragment from pUC19 containing the ampicillin resistance gene and E. coli of replication; an 20 about 1000-bp PvuII to BgIII fragment from pRK248cIts (available from American Type Culture Collection, Rockville, MD) containing lambda transcriptional regulatory regions (including the gene encoding cI^{ts} , the promoter p_R , and a sequence encoding 22 amino acids of the cro protein); an about 60-bp Bg/II to XbaI fragment from pGEMEX-1 25 (available from Promega Corp., Madison WI) which contains the T7 promoter; an about 166-bp XbaI to EcoRI fragment from pRSET-B (available from Invitrogen Corp., San Diego CA) which contains sequences encoding the T7-S10 translational enhancer, the His, fusion, the 14-amino acid S10 leader fusion, and an enterokinase cleavage site as well as the multiple cloning site; and an about 210-bp EcoRI to AatII fragment containing synthetic translational and transcription termination signals including the T₁ translation terminators in all three reading frames, an RNA stabilization sequence from

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Bacillus thurengiensis crystal protein and the T_2 rho-independent transcription terminator from the trpA operon.

The resulting recombinant molecules were transformed into *E. coli* to form recombinant cells, using standard techniques as disclosed in Sambrook et al., *ibid*.

The recombinant cells were cultured in shake flasks containing an enriched bacterial growth medium containing 0.1 mg/ml ampicillin and 1% glucose at about 32°C. When the cells reached an OD₆₀₀ of about 0.6, expression of the Toxoplasma antigen was induced by quickly adjusting the temperature to 42°C and continuing cultivation of the cells for about 2 hours. Protein production was monitored by SDS PAGE of recombinant cell lysates, followed by immunoblot analysis using standard techniques as described herein and as known in the art. The results of these assays are summarized in Table 6

The antisera used to originally isolate each Toxoplasma-specific nucleic acid molecule (i.e., either antiserum 4604, or antiserum 4499) was used to identify recombinant proteins in *E. coli* extracts as follows. The material in crude extracts from *E. coli* were separated by running 5 µg protein per lane on a 12-well 10% Tris-glycine SDS-P VOI gel at 200 volts for 1 hour, and then transferred to nitrocellulose membranes by standard methods. After transfer, the membranes were blocked in 5% (w/v) dry milk for 1 hr at 3.7 °C. The membranes were then incubated with a 1:200 dilution in Tris buffered saline of the sera originally used to select the nucleic acid molecule encoding. Toxoplasmal pecific portion of the fusion protein. After 1 hr incubation at room temperature (the blots were washed, and antibody binding resolved using a secondary antibody bound to a substrate for a color indicator. Using the original selecting antibody membrablet analysis of *E. coli* lysates identified fusions proteins at or near the predicted molecular weight of the recombinant fusion protein. The results of these assays are summarized in Table 6.

Historic tagged fusion proteins were purified from cell lysates as follows. Cell cultures containing nucleic acid molecules of the present invention inserted into either pTrcHisBor \(\lambda\) (RO) were grown to an OD₆₀₀ of approximately 0.4 to 0.5. The cultures were induced with IPTG, and the cells harvested 4 hours later. Ten ml of cell culture was centrifuged at 3000 rpm on a table top centrifuge and the protein isolated according

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to the manufacturer's instructions using a Ni-NTA Spin Kit (available from Qiagen Inc.). Protein purification was monitored by SDS PAGE followed by Coomassie Blue staining of the column eluate fractions. Recombinant cells including recombinant molecules 4499-9, 4604-2, 4604-3, 4604-54, and 4604-69 produced proteins that were able to bind to a T7 tag monoclonal antibody (available from Novagen Inc., Madison, WI) directed against the fusion portion of the recombinant fusion protein.

Recombinant nucleic acid molecules and protein molecules including sequences encoding *T. gondii* antigenic proteins and sequences from the vector pTrcHisB:

Recombinant nucleic acid molecules including sequences encoding T. gondii antigenic proteins and sequences from the vector pTrcHisB were produced as described in Example 2. In brief, T. gondii DNA fragments in λ gt11 were PCR amplified from nucleic acid molecules herein designated 4604-62, 4604-63, R8050-2, R8050-5, and R8050-6, using the λ gt11 forward and reverse primers herein described. The resulting recombinant molecules were transformed into E. coli to form recombinant cells 4604-62, 4604-63, R8050-2, R8050-5, and R8050-6. Immunoblot analysis of the recombinant cell lysates using a T7 tag monoclonal antibody (available from Novagen Inc., Madison, WI) directed against the fusion portion of the recombinant Toxoplasma fusion protein was used to confirm the expression of the fusion proteins and to identify their size. Of the six nucleic acid molecules selected by immunoscreening with rabbit anti-ICG antiserum that were subcloned into the expression vector pTrcHisB, 15(4604-62, R8050-2, and R8050-5) were positive by this immunoblot assay. The results of these assays are summarized in Table 6.

Example 5:

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This Example discloses a method of isolation of *T. gondii* nucleic acid molecules encoding immunogenic *T. gondii* proteins recognized by cat antisera raised against infected cat gut. This Example further discloses recombinant nucleic acid molecules, proteins and cells of the present invention.

Preparation of cat antibody against infected cat gut: Preparation of infected cat gut material and production of anti-ICG antisera in cats was performed essentially as herein described for production of rabbit anti-ICG antiserum. Polyclonal cat antisera against infected cat gut (ICG) antigens (also herein referred to as anti-ICG antiserum or

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antisera, or anti-ICG antibody) were prepared by immunization of cats as follows. Three cats were injected with cat gut material. One cat (#AME5) was injected with material from uninfected cat gut material and two cats (#AMI4, #AMX1) were injected with material from infected cat gut preparations. The same injection, boost and bleed regimen and antigen preparation were used for cats as was used for rabbits, described above. Like the rabbit antisera, the cat antisera were preabsorbed to uninfected cat gut material prior to use in immunoscreening.

Anti-sera AMI was used to isolate nucleic acid molecules herein designated AMI-23, AMI-24, AMI-28, and AMI-47 as follows: *E. coli* Y1090 was infected with approximately 5X10⁶ PFU and then evenly spread on 10 LB-amp agarose culture plates. The rest of the screening procedure was as described for immunoscreening with antisera Q4-1959 (Example 2), with the following exceptions: the primary antibody was used at a 1:200 dilution, and the secondary antibody was a 1:1000 dilution of AP-conjugated goat anti-cat IgG. Of the 5X10⁶ plaques screened in this manner, 6 nucleic acid molecules capable of expressing proteins recognized by antisera AMI were plaque purified.

Anti-sera AMX/I was used to isolate nucleic acid molecules herein designated AMX/I-5, AMX/I-6, AMX/I-7, AMX/I-9, and AMX/I-10 as follows: *E. coli* Y1090 was infected with approximately 5X10⁶ PFU and then evenly spread on 12 LB-amp agarose culture plates. The rest of the screening procedure was as described for immunoscreening with antisera Q4-1959 (Example 2), with the following exceptions: the primary antibody was used at a 1:200 dilution, and the secondary antibody was a 1:1000 dilution of AP-conjugated goat anti-cat IgG. Of the 5X10⁶ plaques screened in this manner, 6 nucleic acid molecules capable of expressing proteins recognized by antisera AMX/I were plaque purified. The results of this immunoscreen are summarized in Table 7.

Table 7

Nucleic Acid Molecules Selected by Cat Serum Specific to Infected Cat Gut

		_	_	-	_	_					,
REACTIVITY	<u>S</u>		+		 - 						
REAC	SERUM		2	Q.	2	9	9	9	9	2	9
oDVAC	IN VIVO		QN	S	2	2	S	S	£	2	2
νQd	IN VITRO IN VIVO		QN	Q.	Q	QN	QN	Q	9	9	QN
NOIS	λCRO		+	+	+	ND	+	QN	QN	QN	ND
EXPRESSION	pTrCHIS		+	QN	QN	+		ND	+	ON	+
	8Z		+	+	+	+	•	•	2+	2+	•
DETECTION	7.2		+	2+	•	+			+	+	+
DETE(DCG		,			•	+	+	•	•	1
	ICG		+	2+	2+	2+	+	+	+	+	•
ORIGINAL SEQ ID NO DESIGNATION			AMX/I-5	AMX/I-6	AMX/I-7	AMX/I-9	AMX/I-10	AMI-23	AMI-24	AMI-28	AMI-47
SEQ ID NO			54	22	25	29	61	63	92	29	89

Table 7 Legend: See Legend for Table 2.

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Selected nucleic acid molecules identified by screening for the expression of proteins recognized by cat anti-ICG antisera were subcloned and sequenced as described in Example 2.

Sequence analysis of nucleic acid molecules selected for expression of proteins recognized by cat anti-ICG antisera AMI and AMX/I:

The nucleic acid molecules isolated using antisera AMI or AMX/I were sequenced as described above. BLASTn and BLASTp homology searches were performed on these sequences using the NCBI GenBank™ non-redundant (nr) nucleotide (n) and amino acid (p) databases, and the dbEST (est) database as described above. The results of these searches are summarized in Table 3.

The sequence data described above were used to design unique primers specific to each nucleic acid molecule of the present invention. These primer sequences are listed in Table 4. The unique primers listed in Table 4 were used in reverse transcriptase-polymerase chain reaction (RT-PCR) assays to assess the expression of the particular nucleic acid sequence in ICG, bradyzoites and tachyzoites. The results of these assays are summarized in Table 7.

T. gondii nucleic acid molecules encoding immunogenic T. gondii proteins isolated by immunoscreening with cat anti-ICG antiserum (antiserum AMI or AMX/I) were subcloned into either or both of two expression vectors: pTrcHisB or Prcro/T2ori/RSET-B (as described above). Expression of the fusion proteins from these vectors, and purification of their expressed fusion proteins, were as described above.

Recombinant nucleic acid molecules, protein molecules and cells including sequences encoding *T. gondii* antigenic proteins and sequences from the vector Prero/T2ori/RSET-B:

Recombinant molecules containing *T. gondii* nucleic acid molecules operatively linked to lambda phage transcriptional control sequences and to a fusion sequence encoding a poly-histidine segment in the vector Prcro/T2ori/RSET-B, were produced essentially as described above, resulting in the production of recombinant molecules. The resulting recombinant molecules were transformed into *E. coli* to form recombinant cells using standard techniques as disclosed in Sambrook et al., *ibid*. Assays for the

expression of an immunogenic *T. gondii* fusion protein by these cells were performed as described above, and the results are summarized in Table 7.

Recombinant nucleic acid molecules, protein molecules and cells including sequences encoding *T. gondii* antigenic proteins and sequences from the vector pTrcHisB:

Recombinant nucleic acid molecules including sequences encoding *T. gondii* antigenic proteins and sequences from the vector pTrcHisB were produced as described in Example 2. In brief, *T. gondii* DNA fragments in λ gt11 were PCR amplified from nucleic acid molecules herein designated AMX/I-5, AMX/I-9, AMI-24 and AMI-47 using the λ gt11 forward and reverse primers herein described. The resulting recombinant molecules were transformed into *E. coli* to form recombinant cells AMX/I-5, AMX/I-9, AMI-24 and AMI-47. Immunoblot analysis of the recombinant cell lysates using a T7 tag monoclonal antibody (available from Novagen Inc., Madison, WI) directed against the fusion portion of the recombinant Toxoplasma fusion protein was used to confirm the expression of the fusion proteins and to identify their size. The results of this immunoblot analysis are summarized in Table 7.

Example 6:

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This Example discloses a method of isolation of *T. gondii* nucleic acid molecules encoding immunogenic *T. gondii* proteins recognized by cat immune sera. This

Example further discloses recombinant nucleic acid molecules, proteins and cells of the present invention.

Production of cat immune sera:

Eight specific-pathogen free (SPF) cats (available from Liberty Laboratories, Liberty Corners, NJ), ages 8-10 months, were randomly assigned to two groups; Group 1, n = 5 and Group 2, n = 3 (the uninfected control group). Before the initiation of any studies with these animals, serum samples were taken from each and tested for reactivity to solubilized tachyzoites. Each animal was seronegative for *T. gondii* by standard Western and ELISA analysis using solubilized tachyzoites as the antigen. This serum also served as the pre-bleed in subsequent studies. Feces from each animal were analyzed for the presence of shed *T. gondii* oocysts using flotation by sugar solution centrifugation followed by microscopic examination. Food was removed from both

groups fourteen hours prior to Day 0, and on the day prior to all sample collections. On Day 0 the cats in Group 1 were orally inoculated by syringe at the back of the throat with 1000 mouse brain derived *T. gondii* tissue cysts of the Mozart strain. This strain represents an isolate from a cat which presented with Toxoplasmosis at the Veterinary Teaching Hospital, Colorado State University, in 1992. The Group 2 cats were not infected.

The Group 1 cats were housed in individual stainless steel cages in an infectious disease isolation unit. The feces from each animal were collected every day for the first fourteen days post infection (PI) and weekly thereafter until parasite challenge. The feces were analyzed for the presence of shed *T. gondii* oocysts. Five milliliters of whole blood was collected from each animal by jugular venipuncture on the following days post primary infection: 3, 7, 10, 14, 21, 28, 42, 56, 70, 84, 112, 140, 143, 147, 154, 161, 168, and 182.

On day 140 post primary infection, all Group 1 cats were orally challenged with 1000 mouse brain-derived tissue cysts of the Mozart strain. Fecal samples were collected and monitored for the excretion of oocysts for thirty days post challenge (PC). The cats were then bled as before on days: 3, 7, 14, 21, 28, and 42 post challenge.

In addition to the serum samples collected on the bleed dates, both salivary secretions and intestinal secretions were obtained at weeks 0, 1, 2, 4, 8, 10, 16, 20, 21, 22, 23, 24, and 26. These samples were obtained by first anesthetizing each animal with an injection of thiobarbiturate, then intubating the animals and maintaining them with halothane and oxygen. Approximately 0.1 ml of saliva was collected into an equal volume of 0.1 M EDTA. The intestinal secretions were obtained from the upper portion of the small intestine using an endoscope fitted with medical tubing which allowed suction of intestinal fluid. Intestinal secretions were diluted 1:1 with sterile 0.9% NaCl and centrifuged at 10,000 X g for 5 minutes in an Eppendorf centrifuge. The secretions were stored at -70°C until use. Pooled secretions included equal aliquots from all five immune animals from week 20 through 26 post infection. These pooled secretions were used to test the reactivity of intestinal secretions from immune cats to proteins expressed by nucleic acid molecules of the present invention.

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All Group 1 animals shed oocysts in their feces during the primary infection and all seroconverted as assessed by Western blot analysis using tachyzoite lysates as the antigen. None of these animals shed oocysts when challenged, and were therefore considered immune. The sera from the immune animals was pooled, and is referred to herein as Mozart II antiserum or antisera, or as immune antiserum or antisera.

Mozart II antisera was used to isolate nucleic acid molecules herein designated 4CQA-7, 4CQA-11, 4CQA-19, 4CQA-21, 4CQA-22, 4CQA-24, 4CQA-25, 4CQA-26, 4CQA-27, and 4CQA29 as follows: *E. coli* Y1090 was infected with approximately 8.3X10⁵ PFU and then evenly spread on 13 LB-amp agarose culture plates. The rest of the screening procedure was as described for immunoscreening with antisera Q4-1959 (Example 2), with the following exceptions: the primary antibody was used at a 1:80 dilution, and the secondary antibody was a 1:50 dilution of monoclonal mouse anti-cat α chain (available from Serotec, Oxford, England) and the tertiary antibody was a 1:1000 dilution of AP-conjugated goat anti-mouse IgG (Kirkegaard Perry Laboratories). Of the 8.3X10⁵ plaques screened in this manner, 13 nucleic acid molecules capable of expressing proteins recognized by Mozart II antisera were plaque purified. The results of assays to characterize these nucleic acid molecules are summarized in Table 8.

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Table 8

Nucleic Acid Molecules Selected with Immune Cat Sera in Screens II and III

VITY	IS		+	+		+			,		,				+									
REACTIVITY	SERUM	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
AC	ONIN NI	+	+	QN	QN	QN	QN	QN	QN	QN	ΟN	QN	QN	QN	QN	DN	QN	ON	QN	QN	QN	QN	Q	Q
pDVAC	IN VITRO	+	+	QN	QN	+	QN	ND	ND	ND	QN	QN	QN	QN	QN	Q	Ð	QN	QN	ND	QN	QN	Q	2
EXPRESSION	ICRO	QN	ND	QN	QN	ND	QN	QN	Q	Q	QN	Q	QN	ND	ND	QN	9							
EXPRE	pTrCHIS	+	+	+	QN	•	•	+	•	•	٠	•	+	•	QN	2	2	2	Q	Q	ND	R	Q	2
	BZ	3+	+	+	QN	2+	+	+	2+	3+	3+	+	+	2+	+	2	•	+	Q	•	+	+	Q	+
TION	7.2	+	2+	+	ND	+	+	3+	3+	2+	2+	+	+	+	+	ND	•	+	ND	,	+	+	ΩN	+
DETECTION	nce	•	•	•	QN	-	•	•		•	•	+	•	•	-	ON	•	•	•	•	•	•	•	
	901	2+	+	+	ND	2+	+	3+	+	+	+	+	+	+	+	QN	•	+	+	•	+	+	+	+
ORIGINAL DESIGNATION		Tg-41	Tg-45	Tg-50	4CQA-7	4CQA-11	4CQA-19	4CQA-21	4CQA-22	4CQA-24	4CQA-25	4CQA-26	4CQA-27	4CQA-29	M2A-1	M2A-2	M2A-3	M2A-4	M2A-5	M2A-6	M2A-7	M2A-11	M2A-16	M2A-18
SEQ ID NO		-	3	2	82	85	87	89	91	93	95	97	66	101	109	111	113	115	117	119	121	123	125	127

Table 8

88555588 Nucleic Acid Molecules Selected with Immune Cat Sera in Screens II and III S 5 5 CH 5558 8 5 5 5 5 5 2 5 5 Ģ ₹ **5** MS 21 95 a 22 95 a 24 95 a 34 M2A 25 1.12A.20 M2A-19 131 132 132 132 141 129

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Table 8 Legend: See Legend for Table 2.

M2A-29

Page 2 of 2

In addition to the immunoscreen described above, Mozart II antisera was used in another immunoscreen to isolate nucleic acid molecules herein designated M2A1, M2A2, M2A3, M2A4, M2A5, M2A6, M2A7, M2A11, M2A16, M2A18, M2A19, M2A20, M2A21, M2A22, M2A23, M2A24, M2A25, and M2A29 as follows: *E. coli* Y1090 was infected with approximately 1X106 PFU and then evenly spread on 10 LB-amp agarose culture plates. The rest of the screening procedure was as described for immunoscreening with antisera Q4-1959 (Example 2), with the following exceptions: the primary antibody was used at a 1:50 dilution, and the secondary antibody was a 1:200 dilution of AP-conjugated goat anti-cat IgA (available from Bethyl Laboratories Inc., Montgomery, Texas). Of the 1X106 plaques screened in this manner, 18 nucleic acid molecules capable of expressing proteins recognized by Mozart II antisera were plaque purified. The results of assays to characterize these nucleic acid molecules are summarized in Table 8.

Mozart II antisera was also used in yet another immunoscreen to isolate nucleic acid molecules herein designated Tg-41, Tg-45, and Tg-50 as follows: *E. coli* Y1090 was infected with approximately 1X10⁶ PFU and then evenly spread on 12 LB-amp agarose culture plates. The rest of the screening procedure was as described for immunoscreening with antisera Q4-1959 (Example 2), with the following exceptions: the primary antibody was used at a 1:50 dilution, and the secondary antibody was a 1:200 dilution of AP-conjugated goat anti-cat IgA Fc. Of the 1X10⁶ plaques screened in this manner, 4 nucleic acid molecules capable of expressing proteins recognized by Mozart II antisera were plaque purified. The results of assays to characterize these nucleic acid molecules are summarized in Table 8.

Selected nucleic acid molecules identified by screening for the expression of proteins recognized by Mozart II (immune) antiserum were subcloned and sequenced as described in Example 2.

Sequence analysis of nucleic acid molecules selected for expression of proteins recognized by Mozart II (immune) antiserum:

The nucleic acid molecules isolated using Mozart II (immune) serum were sequenced as described above. BLASTn and BLASTp homology searches were performed on these sequences using the NCBI GenBankTM non-redundant (nr)

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nucleotide (n) and amino acid (p) databases, and the dbEST (est) database as described above. The results of these searches are summarized in Table 3. Nucleic acid molecule M2A3 was sequenced again and some changes found between the first and second sequence. The resequenced nucleic acid molecule is referred to herein as M2A3-a. In addition, nucleic acid molecule M2A18 was sequenced again and some changes found between the first and second sequence. The resequenced nucleic acid molecule is referred to herein as M2A18-a.

The sequence data described above were used to design unique primers specific to each nucleic acid molecule of the present invention. These primer sequences are listed in Table 4. The unique primers listed in Table 4 were used in reverse transcriptase-polymerase chain reaction (RT-PCR) assays to assess the expression of the particular nucleic acid sequence in ICG, bradyzoites and tachyzoites. The results of these assays are summarized in Table 8.

Recombinant nucleic acid molecules, protein molecules and cells including sequences encoding *T. gondii* antigenic proteins and sequences from the vector pTrcHisB:

Recombinant nucleic acid molecules including sequences encoding T. gondii antigenic proteins and sequences from the vector pTrcHisB were produced as described in Example 2. In brief, T. gondii DNA fragments in λ gt11 were PCR amplified from nucleic acid molecules herein designated 4CQA-11, 4CQA-19, 4CQA-21, 4CQA-22, 4CQA-24, 4CQA-25, 4CQA-26, 4CQA-27, 4CQA-29, Tg-41, Tg-45, and Tg-50 using the λ gt11 forward and reverse primers herein described. The resulting recombinant molecules were transformed into E. coli to form recombinant cells. Immunoblot analysis of the recombinant cell lysates using a T7 tag monoclonal antibody (available from Novagen Inc., Madison, WI) directed against the fusion portion of the recombinant Toxoplasma fusion protein was used to confirm the expression of the fusion proteins and to identify their size. The results of this immunoblot analysis are summarized in Table 8.

Example 7:

This Example discloses a method of isolation of *T. gondii* nucleic acid molecules encoding immunogenic *T. gondii* proteins recognized by cat immune sera enriched for

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antibodies to gametogenic stages (herein referred to as absorbed immune sera or serum). This Example further discloses recombinant nucleic acid molecules, proteins and cells of the present invention.

Production of cat immune sera enriched for antibodies to gametogenic stages:

Sera from cats which were infected and then subsequently challenged with mouse brain-derived tissue cysts were tested for reactivity to extracts of infected cat gut material by Western blot analysis. Sera from one specific cat, designated Queen 2, demonstrated reactivity to particular ICG sections in which the presence of *T. gondii* had been shown by immunofluorescence assay. Queen 2 was originally infected with 100 mouse brain-derived tissue cysts, did not shed oocysts, and seroconverted to positive for tachyzoite antigens by day 39 post-infection. This sera was highly reactive to the asexual stage, tachyzoites. Therefore, to enhance the utility of this sera as a reagent for detection of gametogenic proteins, this sera was used in conjunction with a western blot of infected cut intestinal cell lysates to obtain a fraction enriched in antibody reactive to the gametogenic proteins. The enrichment of the Queen 2 sera (also referred to herein as Q2 sera) was performed as follows:

A 12% SDS-PAGE gel was prepared according to standard methods (Laemmli, 1970, Nature 227, 680-685). 1000 µg of solubilized ICG protein, prepared as described above, was loaded on 20 x 20 x 0.1 cm gel and run at 8V/cm for 5 hours. Toxoplasma tachyzoite (17) antigen, prepared from solubilized tachyzoites, was used as a control. Separated proteins were transferred to nitrocellulose according to standard procedures for western borting. After transfer, the nitrocellulose filter was blocked with 4% (w/v) dry milk row term PBS (pH 7.5), and incubated with a 1:200 dilution of immune cat (Queen 2) in the crum at room temperature for 5 hours with gentle shaking. The filter was then washed with PBS (pH 7.5). After washing, a 0.5 cm strip was cut off the end of the filter and incubated with a 1:1000 dilution of alkaline phosphatase labeled goat anti-cat IgG antibody at room temperature for 1 hour. The strip was stained with 5-bromo-4-chloro-3-indolylphosphate p-toluene salt/nitroblue tetrazolium chloride substrates (BCIP/NBT) cavailable from Gibco/BRL). The areas of the gel that stained with BCIP/NBT substrates represented ICG protein bands which were recognized by IgG antibodies in immune cat serum.

The regions of interest that were visualized on the BCIP/NBT-stained end strip were cut from the remainder of the filter, and the bound antibody eluted with 0.1 M glycine (pH 2.8), 1 mM EDTA at room temperature for 10 minutes. The antibody in glycine was neutralized with 10 mM Tris (pH 9.0), 0.02% NaN₃ was added, and the solution was stored at 4° C. The purified antibody was analyzed by Western blot of ICG to monitor successful recovery of the eluted antibody, verifying recovery of antibody that reacted with the appropriate molecular weight region of the ICG western blot. This antibody preparation is referred to herein as absorbed immune serum or sera.

The absorbed immune serum was used to isolate nucleic acid molecules herein designated Q2-4, Q2-9, Q2-10, and Q2-11 as follows: *E. coli* Y1090 was infected with approximately 3.2X10⁵ PFU and then evenly spread on 8 LB-amp agarose culture plates. The rest of the screening procedure was as described for immunoscreening with antisera Q4-1959 (Example 2), with the following exceptions: the primary antibody was used at a 1:200 dilution, and the secondary antibody was a 1:1000 dilution of AP-conjugated goat anti-cat IgG. Of the 3.2 x 10⁵ plaques screened in this manner, 4 nucleic acid molecules capable of expressing proteins recognized by absorbed immune serum were plaque purified. The results of assays to characterize these nucleic acid molecules are summarized in Table 9.

Table

Nucleic Acid Molecules Selected with Absorbed Immune Sera

BZ pTrCHIS \(\text{\chick} \) \(ORIGINAL		DETECTION	NOIL		EXPRESSION	NO	pDVAC	AC	REACTIVITY	IIVITY
Q2-4 2+ - + 2+ ND + ND Q2-9 + - + + - - ND Q2-10 + - + + ND + ND Q2-11 - - + ND + ND	SEG ID NO			930	71	BZ	pTrCHIS		IN VITRO	IN VIVO	SERUM	SI
Q2-4 2+ - + 2+ ND + ND Q2-9 + - + + - - ND Q2-10 + - + + ND + ND Q2-11 - - + + ND + ND												
Q2-4 2+ - + 2+ ND + ND Q2-9 + - + + - - ND Q2-10 + - + + ND + ND Q2-11 - - + + ND + ND												
Q2-4 2+ - + 2+ ND + ND Q2-9 + - + + - - ND Q2-10 + - + + ND + ND Q2-11 - - + + ND + ND										2		
Q2-9 + + + - ND O2-10 + ND + ND O2-11 ND O2-11		V 60	5+	٠	+	2+	2	+	ם ב	ND	٠	
Q2-9 + - + + ND + ND + ND C2-10 + ND + ND C2-11 C2-1	מ	4-75								2	4	
Q2-10 + ND + ND O2-11 C2-11 + ND O2-11	÷	02.0	+	•	+	+		•	מא	2	,	
Q2-10 + + ND + ND + ND C2-11	2	W6-3					٢		CN	2	+	
Q2-11 + + ND + ND	15	102-10	+	,	+	+	2	+	בַּ	2		·
	2	42-10					١	4	S	Ş	+	٠
	17	02-11	,	,	+	+	אַ	-		2		
	-	13										

Table 9 Legend: See Legend for Table 2.

Selected nucleic acid molecules identified by screening for the expression of proteins recognized by absorbed immune serum were subcloned and sequenced as described in Example 2.

Sequence analysis of nucleic acid molecules selected for expression of proteins recognized by absorbed immune serum:

The nucleic acid molecules selected for expression of proteins recognized by absorbed immune serum were sequenced as described above. BLASTn and BLASTp homology searches were performed on these sequences using the NCBI GenBankTM non-redundant (nr) nucleotide (n) and amino acid (p) databases, and the dbEST (est) database as described above. The results of these searches are summarized in Table 3. Nucleic acid molecule Q2-9 was sequenced again and some changes found between the first and second sequence. The resequenced nucleic acid molecule is referred to herein as Q2-9-a.

The sequence data described above were used to design unique primers specific to each nucleic acid molecule of the present invention. These primer sequences are listed in Table 4. The unique primers listed in Table 4 were used in reverse transcriptase-polymerase chain reaction (RT-PCR) assays to assess the expression of the particular nucleic acid sequence in ICG, bradyzoites and tachyzoites. The results of these assays are summarized in Table 9.

Recombinant nucleic acid molecules, protein molecules and cells including sequences encoding *T. gondii* antigenic proteins and sequences from the vector Prcro/T2ori/RSET-B:

Recombinant molecules containing *T. gondii* nucleic acid molecule operatively linked to lambda phage transcriptional control sequences and to a fusion sequence encoding a poly-histidine segment in the vector Prcro/T2ori/RSET-B, were produced essentially as described above, resulting in the production of recombinant molecule. The resulting recombinant molecules were transformed into *E. coli* to form recombinant cells using standard techniques as disclosed in Sambrook et al., *ibid*. Immunoblot analysis of expression of immunogenic *T. gondii* proteins by these recombinant cells is summarized in Table 9.

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Example 8:

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This Example describes the construction of several cDNA expression libraries of the present invention.

A *T. gondii* tachyzoite cDNA expression library, a *T. gondii* infected cat gut (ICG) cDNA library (constructed from seven day post infection infected cat gut material, which is a mix of both cat intestinal cDNA and *T. gondii* gametogenic cDNA), and an uninfected cat gut (UCG) cDNA expression library were from total RNAs as follows:

Isolation of Total RNA From Tachyzoites: Total RNA from tachyzoites was prepared using Tri-ReagentTM (available from Molecular Research Center, Inc, Cincinnati, Ohio) according to the manufacturer's directions. Briefly, 4 X 10⁹ tachyzoites were resuspended in 6 ml of TriReagent with a syringe and 18 gauge needle. Successive triturations were made with 20 gauge and 22 gauge needles. A volume of CHCl₃ equal to one-fifth the original volume of TriReagent[®] was added and the mixtures were shaken for 15 seconds. The aqueous and organic phases were then separated by centrifugation. Total RNA was recovered from the aqueous phase by precipitation in isopropanol.

PolyA⁺ RNA was isolated from total RNA using Pharmacia mRNA purification kit (available from Pharmacia Biotech Inc., Piscataway, NJ).

Isolation of Total RNA from Other Sources: The method of isolation of total RNA from various tissues was the same for all tissues. The only variable was the starting material. For example, to obtain RNA from infected cat gut (ICG) or uninfected cat gut (UCG), the epithelial layer of a fifteen square centimeter section of gut was scraped into 6 ml of Tri-Reagent and processed as described above. RNA from mouse was obtained from 1 gm of mouse brain and treated with Tri-Reagent as described above. RNA from bradyzoites was obtained from 7,000 tissue cysts propagated in mouse brain, obtained as described, and treated with Tri-Reagent as described above.

Poly A⁺ mRNA was isolated from total RNA using Pharmacia mRNA purification kit (available from Pharmacia Biotech Inc., Piscataway, NJ).

Preparation of λ cDNA libraries:

The ZAP-cDNA® synthesis kit (available from Stratagene) was used according to manufacturer's instructions to synthesize cDNA. Briefly, 5 or 10 μg of PolyA⁺ mRNA (prepared as described above) was reverse transcribed using Superscript reverse transcriptase and 0.6 mM dGTP, dATP, dTTP, and 0.3 mM 5-methyl dCTP and 1.4 µg of oligo dT linker primer supplied with the ZAP-cDNA® Synthesis Kit. The second strand was made by digesting the RNA template with RNaseH and priming second strand synthesis with DNA polymerase I. The cDNA was then ligated into the Uni-ZAP® XR lambda insertion vector (available from Stratagene), packaged and amplified to produce tachyzoite and ICG cDNA libraries.

5 μg of polyA+ RNA was used to prepare the ICG cDNA library, and 10 μg of polyA+ RNA was used to prepare the tachyzoite cDNA library. For each library, 100 ng of double stranded cDNA was ligated and packaged and gave approximately 1.5 X 106 unique nucleic acid molecules. The average size of the cloned inserts was 1.9 Kb in the tachyzoite cDNA library, and 2.1 Kb in the ICG cDNA library.

Example 9:

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This Example describes the construction and identification of cDNA sequences encoding near full-length T. gondii nucleic acid molecules encoding immunogenic T. gondii proteins.

Two of the molecular libraries described above were used to isolate near fulllength T. gondii nucleic acid molecules encoding immunogenic T. gondii proteins: the tachyzoite cDNA library and the ICG cDNA library constructed from seven day post infection infected cat gut material.

The general approach to isolating nucleic acid sequences representing full length, or near full-length cDNA sequences was as follows: First, the MacVector DNA analysis program was used to design DNA primers for each of the Toxoplasma sequences cloned in an expression vector as herein described. These primers were then used in a PCR reaction in which the template was either of the Toxoplasma cDNA libraries herein described. The presence of a positive band on an agarose gel following PCR was 30 diagnostic of the presence in the cDNA library of a nucleic acid molecule with homology to the primers. A near full-length cDNA molecule having sequence homology with the

genomic DNA sequence designated Q2-4 was obtained by a direct hybridization screen of the libraries using radiolabeled clone-specific PCR fragments as templates. The isolation of one of these near full-length sequences is herein described in detail as representative of the methods used to isolate all of the near full-length sequences identified by this strategy.

A cDNA sequence representing a near full-length gene having homology to a nucleic acid sequence herein designated Q2-4 (isolated from the Toxoplasma genomic DNA library) was isolated from the infected cat gut (ICG) cDNA library by hybridization screening as follows: *E. coli* Y1090 was infected with approximately 1X10⁶ PFU of the Toxoplasma ICG cDNA library and then plated at a density of about 50,000 plaques per 150 mm agar plate. The resulting plaques were transferred to nitrocellulose filters. The filters were then soaked in denaturing solution (1.5 M NaCl, 0.5 M NaOH) for two minutes, neutralization solution (1.5 M NaCl, 0.5 M Tris, pH 8) for five minutes, and then rinsed several times in 2 X SSC (150 mM NaCl, 15 mM Na citrate, pH 7). The DNA bound to the filters was crosslinked using a Stratalinker[®] UV crosslinker (available from Stratagene) according to the manufacturer's directions.

A radioactive hybridization probe was made by incorporating ³²P into clone-specific template DNA using a Prime-It[®] II random primer labeling kit (available from Stratagene) following the manufacturers directions. The template was a PCR fragment generated by using two primers specific for Q2-4. For each 100 µl reaction, 30 ng of Toxoplasma genomic DNA was PCR amplified using 200 mM of each dCTP, dGTP, dTP, dATP, 200 nM of each specific primer, 2.5 mM MgCl₂, 20 mM Tris pH 8.4, 50 mM KCl, and 2.5 units *Taq* DNA polymerase (available from The Perkin Elmer Corp.) for thirty-five cycles in a Perkin-Elmer Gene Amp PCR System (available from The Perkin Elmer Corp.).

The nitrocellulose filters containing crosslinked DNA were hybridized in 2 X PIPES buffer (10 mM piperazine-N, N'-bis[2-ethanesulfonic acid] (pH 6.5), 400 mM NaCl), 50% formamide, 0.5% SDS, 100 mg/ml denatured salmon sperm DNA and 10⁷ cpm/ml of the radioactive hybridization probe. The filters were incubated with this hybridization solution overnight at 42°C. The next day the filters were washed in 0.1 X

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SSC, 0.1 % SDS and then exposed to X-ray film (available from Kodak, Rochester, NY) in order to visualize positive plaques.

Plaques in the area corresponding to the positive signals were picked into SM buffer (50 mM Tris, pH 7.5, 100 mM NaCl, 8 mM MgSO₄, and 0.01% gelatin) and the phage replated at a lower density. The same screening procedure was repeated three or four times until a pure plaque corresponding to a full length cDNA nucleic acid sequence representing Q2-4 was isolated.

After plaque purification, the nucleic acid molecules were mapped and the areas of interest sequenced using primers specific to the original clone, long fragment PCR, and cycle sequencing of the large fragments.

Example 10:

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This Example describes the expression in a eucaryotic cell of nucleic acid molecules encoding immunogenic *T. gondii* proteins, and DNA vaccination with nucleic acid molecules encoding immunogenic *T. gondii* proteins.

Cloning into a eucaryotic expression vector(pDVacI):

Inserts from eight clones (OC-2, OC-13, OC-14, OC-22, Tg-41, Tg-45, Tg-50, 4CQA-11) were ligated into the pDVacI expression vector. This vector contained a eucaryotic promoter from cytomegalovirus (CMV), followed by the start codon and signal sequence for a mouse kappa immunoglobulin gene. An *EcoR* I site was inserted in frame downstream to the signal sequence. This allowed the insertion of *Eco RI* fragments directly from the original lambda phage. The nucleic acid molecules produced by insertion of nucleic acid molecules encoding immunogenic *T. gondii* proteins into pDVacI are referred to herein as pDVacI:Toxoplasma nucleic acid molecules. If the *EcoR* I inserts represent nucleic acid sequence that is entirely open reading frame, then the protein product expressed from these inserts may be in frame with a C-terminal fusion consisting of both a poly histidine track and amino acid sequence representing an epitope from the human *myc* gene as a reporter sequence. The N-terminal fusion adds 49 amino acids, or about 5.4 kD to the protein encoded by the *T. gondii* nucleic acid molecule, and the C-terminal fusion adds 38 amino acids, or about 4.2 kD, to the fusion protein.

Expression in vitro:

WO 99/32633

Direct sequencing of the inserts in each plasmid confirmed the production of eight different pDVacI:Toxoplasma nucleic acid molecules. DNA from these molecules was then tested for eukaryotic expression of antigenic *T. gondii* proteins by transfecting BHK cells *in vitro* with DNA isolated from the pDVacI:Toxoplasma nucleic acid molecules. Analysis of the eukaryotic expression products of the pDVacI:Toxoplasma nucleic acid molecules was done by western blot on cell lysates and on supernatants from the transformed BHK cells. Either a monoclonal reactive with the *myc* epitope or antibody specific to each clone was used as the primary antibody. Seven out of the eight plasmid constructs expressed a protein *in vitro*. See Table 10.

Table 10

Analysis of Clones in Eucaryotic Expression Vector and DNA Vaccination

	Sizo (KD) Expressed in		Expression <i>in vitro</i>	n in vitro	Sero- conversion
Clone	pDVac	EU / ug DNA*	Pellot	Super	(# of Mice)**
OC-2	40	0.3 / 0.4	+	+	5/2/2
OC-13	38	0 / 0.23	+	+	0/0/4
OC-14	32	7.7 / 3.8	ı	ı	*
OC-22	40	0.5 / 0.44	+	+	4/5/5
Tg-41	33	23 / 1.8	+	+	0/1/5
Tg-45	26	0/0	+	+	3/2/2
Tg-50	55	4.0 / 4.0	+	+	5/5/5
4cqa-11	25	0.95 / 5.3	+	+	0/0/0

Table 10 legend:

(*) The first and second numbers represent the endotoxin units (EU)/ug of DNA of mice that sero-converted at the 4, 7, and 9 week bleeds, respectively, out of the group for the first and second immunizations respectively. (**) The numbers represent the # of five that were injected. (***) Antigen for Nt4 protein was not available to analyze for these sera samples.

Expression in vivo:

100 ug of each pDVacI:Toxoplasma nucleic acid molecule was injected intradermally into five mice. The administrations were at day zero and week five; bleeds were collected at weeks four, seven and nine. The mouse sera were used to determine if the DNA vaccination with each clone elicited a serological response to the cloned fusion protein. This was measured by western blot analysis with the protein expressed in the BHK lysates. Six of the eight clones induced antibodies in mice by week nine, see Table 10.

Reactivity of antibody raised against recombinant OC-1 protein:

Purified recombinant protein expressed by an expression vector containing the nucleic acid sequence referred to as OC-1 (SEQ ID NO:70) was used to immunize mice and rabbits by methods well known in the art. The animals were bled, and serum collected used in immunofluorescence assays against infected and uninfected cat gut tissue. The results of these assays showed that antibody raised, in mice and rabbits, to recombinant OC-1 protein bound to most of the enteroepithelial stages in the infected cat gut. The antiserum did not react with uninfected cat gut.

Example 11:

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This example describes the construction of a Toxoplasma gondii EMBL3 genomic library from tachyzoites grown in tissue culture. This Example further describes isolation of near full-length nucleic acid molecules encoding stage specific *T. gondii* antigenic proteins.

An EMBL3 library of Toxoplasma genomic DNA was constructed using standard molecular cloning methods, well known to those skilled in the art of cloning (see, for example, Sambrook, *et al.*, *ibid.*). In brief, Toxoplasma genomic DNA, prepared from tachyzoites as herein described, was partially digested with *Sau*3A I, using a series of different ratios of units of enzyme to μg of DNA. Digestions were incubated at 37°C for one hour. Ratios of 0.06, 0.03, and 0.015 units of enzyme per μg of DNA produced high molecular weight DNA fragments which were then run on a preparative agarose gel. The fraction of the gel corresponding to DNA in a size range of between 15 and 20 Kb was excised. The DNA fragments were extracted from the gel, and the amount of extracted DNA quantitated. The EMBL3 library was then constructed

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using this DNA and the Lambda EMBL3/BamH I Vector Kit (available from Stratagene). The manufacturer's instructions were followed for all cloning steps, and the resulting ligated DNA was packaged using the Gigapack[®] II XL Packaging Extract (available from Stratagene). Packaging and amplification followed the manufacturer's specifications. The resulting library is referred to herein as the EMBL3:Toxoplasma genomic library.

The EMBL3:Toxoplasma genomic library was plated at a density of 50,000 plaques per 150 mM agar plate and the plaques transferred to a nitrocellulose filter. The filters were soaked in denaturing solution (1.5 M NaCl, 0.5 M NaOH) for two minutes, neutralization solution (1.5 M NaCl, 0.5 M Tris, pH 8) for five minutes, rinsed several times in 2 X SSC (150 mM NaCl, 15 mM Na citrate, pH 7), and the DNA crosslinked using a Stratalinker[®] UV crosslinker (available from Stratagene) according to the manufacturer's instructions.

The EMBL3:Toxoplasma genomic library was screened with probes made from PCR amplified nucleic acid molecules isolated by immunoscreening the λ gt11:Toxoplasma genomic library. The primers used to generate these probes were derived using the MacVector Sequence Analysis program and the sequences of nucleic acid molecules encoding T. gondii antigenic proteins isolated from the λ gt11:Toxoplasma genomic library.

The filters were hybridized in 2 X PIPES buffer (10 mM piperazine-N, N'-bis[2-ethanesulfonic acid] (pH 6.5), 400 mM NaCl), 50% formamide, 0.5% SDS, 100 μg/ml denatured salmon sperm DNA (available from Sigma) and 10⁷ cpm/ml of radioactive hybridization probe. The filters were hybridized overnight at 42°C. The next day the filters were washed in 0.1 X SSC, 0.1 % SDS, and then exposed to X-ray film (Kodak).

Plaques in the area corresponding to the positive signals were picked into SM buffer (50 mM Tris, pH 7.5, 100 mM NaCl, 8 mM MgSO₄, and 0.01% gelatin) and the phage replated at a lower density. The same screening procedure was repeated three or four times until a pure plaque hybridizing with a nucleic acid molecule isolated by immunoscreening the λ gt11:Toxoplasma genomic library was isolated. After plaque purification, the nucleic acid molecules were mapped and the areas of interest sequenced

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using primers specific to the original clone, long fragment PCR, and cycle sequencing of the large fragments.

Long fragment PCR was done with a Perkin-Elmer XL PCR kit (available from The Perkin-Elmer Corp., Foster City, CA) as follows: A 100 µl reaction was separated into two layers with a wax bead so one would have a hot-start reaction. The lower layer contained 1 X XL PCR buffer supplied with the kit, 40 pM each of the forward and reverse primers. SC1011 and SC1002, (supplied by the manufacturer with the XL PCR kit, 2.5 mM each dNTP, 1.1 mM Mg(OAc)₂. The upper layer contained 1 X XL buffer, 4 units of r1th DNA polymerase (available from The Perkin-Elmer Corp.) and about 5 ng of the plaque purified EMBL3:Toxoplasma genomic DNA as the template. The reaction was done in a Hybaid thermocycler (available from Hybaid Ltd., Middlesex, UK), and the reaction products were resolved on a 0.6% agarose gel.

Example 12

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This I sample describes the detection of *T. gondii* oocysts in cat feces by PCR amplification of nucleic acid sequences homologous to nucleic acid sequences encoding immunogenic *I. gondii* proteins of the present invention. Specifically, this example describes a rapid PCR dipstick method for the detection of oocysts in feces.

Naive cats were infected per os by 1000 mouse-brain derived tissue cysts of T. gondu strain C at day zero. Feces from each animal were collected, if available, on a daily basis starting at day zero and each day for 19 days post infection (PI). A portion of the feces we created by the standard sugar floatation method (Dubey, J.P., Swan, G. V., and Frenkel J. k. 1972, Journal of Parasitology. 58: 1005-1006) and the oocysts visualized of the amicroscope and counted on a haemacytometer. A portion of each feces was always rended in PBS, vortexed and a small sample obtained by dipping an IsoCode. The stick (available from Schleicher & Schuell, Keene, NH) into the fecal solution. The dipstick was allowed to air dry and then washed in 500 µl of distilled water by vortexing the stick end and water in a tube for 10 seconds. Material adhering to the filter was then cluted in 50 µl of fresh distilled water by heating to 95° C for 30 minutes. The remaining supernatant was then used for standard hot start PCR, according to methods well known in the art, using primers representing DNA sequences from nucleic acid molecules encoding T. gondii antigenic proteins. The results of an

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experiment in which primers derived from nucleic acid molecule OC-2 were used are shown in Table 11. The results of this experiment demonstrated that the PCR detection method was at least as sensitive at detecting oocysts in fecal matter as the conventional floatation method.

PCR Analysis of Cat Feces

	PCR Dipstick Oc2			•	•	•	+	+	+		+	+	+	,	•			•	•	•	•	1
#3515-1	/ Oocysts/gm Float Di		>	0	0	0	1X10e6	5X10e6	1X10e6		2X10e5	7X10e4	0	0	0	0	0	0	0	0	0	0
	Day PI	c	>	-	7	က	4	ည	9	7	∞	တ	10	=======================================	12	13	14	15	16	17	18	19
	PCR Dipstick Oc2		•	•	,	•		+	+	+	+	+	+	+	+	•	•	•			•	•
#3512-1	y <u>Oocysts/gm</u> F	C	>	0	C) C)	1X10e4	3X10e5	1X10e6	1X10e6	1x10e6	1X10e5	1X10e5	0	0	0	0			0	0
	Day PI	•	0	₩.	۰ ،	1 m	> <	ታ ሊ	ာဏ	^	. α	σ	, (7	12	13	14	15	16	17	- 42	9 6
	PCR Dipstick	7	1	ı	ı	ı	•	•	ŀ	,	ı		ı	• (ı							•
•	#3528-U / Oocysts/gm Float Di		c	o 0)	o (0 (0 (o	c	Þ	ć	5 (>	>			c	>			0
	Day Pl		c	> ·	-	7	က	4	വ വ	1 02	_ (∞ (ဘ :	2;	;	77		4 ,	<u>ດ</u> (ا م	7;	<u>ක</u> ව

A series of additional experiments was performed in order to investigate further the PCR dipstick method for the detection of oocyts in feces. In this set of experiments, the following methods were used to produce T. gondii infected cats, and to detect oocysts in the feces of the infected cats. T. gondii C-strain tissue cysts were obtained by orally infecting 6-8 week old Swiss Webster mice with a sub-lethal dose of mouse brain derived tissue cysts. At six weeks post infection, the animals were euthanized with CO, and the brains were removed and placed in 30% Dextran in HBSS (Gibco/BRL). The brains were then homogenized with a Tissuemizer (Tekmar Co., Cincinnati OH) and centrifuged at 5,000 x g's for 10 min at 4°C. The pellet was resuspended in HBSS and the tissue cysts were counted. The tissue cysts were diluted with PBS to the appropriate concentration for oral administration to cats at the back of the throat using a 1ml syringe. A total of twenty cats was used in this study: seventeen were experimentally infected with 1000 tissue cysts and three were used as uninfected controls. All cats were housed in separate cages and feces were collected at the day of infection and daily for the next 21 days. On average there were approximately twelve samples per cat. The fecal samples were stored at 4°C until tested, which was within two weeks of collection.

Conventional quantification of oocysts in feces was based on the sugar flotation method of Dubey and Beattie, 1988, and is described in full as follows. Each fecal sample was weighed and then 2 grams of feces were mixed with 15 ml of sugar solution (53 gm sugar, 100 ml of water). Following solubilization with a tongue depressor, the mixture was passed through two layers of gauze. The filtrate was poured into a 15 ml conical tube and centrifuged at 1,200 x g for 10 minutes. The top 3 ml of the sample was added to 13 ml of sugar solution and centrifuged as above. The top 3 ml of the second flotation was added to 13 ml of water and centrifuged at 1,200 x g for 10 minutes. The resulting oocyst pellet was resuspended in 1ml of water and the oocysts counted using a hemacytometer. Alternatively, the entire fecal sample was solubilized in PBS by adding five ml of PBS per gram of the pre-weighed feces in a 250 ml plastic beaker. After one hour at room temperature, a tongue depressor was used to thoroughly suspend the feces. Five ml of the fecal slurry was added to a 15 ml tube containing 5 ml of 2X sugar solution and inverted several times. The tube was then centrifuged at 1,200

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x g for 10 minutes. The top 3 ml of the sample was subjected to a second sugar flotation, resuspended, and counted as described above.

Analysis of the fecal samples by the PCR dipstick method was performed as follows. One ml aliquots were taken, prior to further processing for floatation, from each of the initial fecal slurries described above. Samples were collected directly onto dipsticks, either by spotting 10 ul onto each dipstick filter or by directly dipping the dipstick into the fecal slurry. The filters were then dried at room temperature and the filter portion of the dipstick was cut off into a sterile 1.5 ml centrifuge tube. The filter was washed with 500 ul of sterile distilled water by vortexing for 8 seconds. The wash was removed and 50 ul of sterile water was added to the tube and adherent oocyst DNA eluted by heating at 95 °C for 1 hour. The filter was removed with a sterile tip and the sample stored (also referred to as the dipstick eluate) at -20 °C.

Primers specific to two T. gondii genes, B1 and OC-2, were used in the amplification reactions. The primers for the B1 gene (Burg, et al., 1989, Journal of Clinical Microbiology, 27: 1787-1792) were B1 forward (5'-GGA ACT GCA TCC GTT CAT GAG-3', herein referred to as SEQ ID NO:332), B1 reverse (5'- TCT TAA AGC GTT CGT GGT C-3', herein referred to as SEQ ID NO:333), and a B1 internal primer (5'-GGC GAC CAA TCT GCG AAT ACA CC-3', herein referred to as SEQ ID NO:334). The T. gondii OC-2 was isolated as herein described. The OC-2-derived primers were OC-2 forward (5'-GCA TCC TTG GAG ACA GAG CTT GAG-3', herein referred to as SEQ ID NO:335), OC-2 reverse (5'-GGG TTC TCT CGC TCA TCT TTC-3', herein referred to as SEQ ID NO:336), and an OC-2 internal primer (5'-AGT CAG AAG CAG TCA AGG C-3' herein referred to as SEQ ID NO:337). The PCR mixture contained 1X PCR buffer (10 mM Tris-HCl₂, 1.5 mM MgCl₂, 50 mM KCl), 0.2 mM deoxynucleoside triphosphates (Perkin-Elmer Cetus Corp., Norwalk, CN), 0.8 uM of each primer, 0.5 U of Gold AmpliTaq™ DNA polymerase (Available from Perkin-Elmer Corp.), and 1 ul DNA template in a total volume of 25 ul. The reaction mixture was denatured at 95°C for 10 minutes, amplified for 42 cycles including a denaturation step at 95°C for 30 seconds, annealing at 58°C for 30 seconds, extension at 72°C for 40 seconds, and a fine tension for 5 minutes at 75°C on an automated DNA thermal

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cycler (Model 9700, Perkin-Elmer, Foster City, CA). PCR products were analyzed by electrophoresis on a 1.5 % agarose gel, stained with ethidium bromide (0.5 ug/ml), and photographed on a UV transilluminator.

Following electrophoresis, the DNA products were denatured in 0.5 N NaOH and
1.5 M NaCl buffer for 30 minutes, transferred to a nylon membrane (Maximum Strength
Nytran Plus, available from Schleicher & Schuell) overnight and cross-linked by
exposure to UV light (UV Stratalinker 1800, available from Stratagene). The filters
were incubated in prehybridization buffer (5 x SSC, 1X Denhardt's reagent, 0.2% SDS,
1 mg/ml sheared DNA) at 42°C for 2 hours and then in hybridization buffer (5 x SSC,
1X Denhardt's reagent, 0.2% SDS, 1 mg/ml sheared DNA) containing 5' γ-32P labeled
oligonucleotide probe at 42°C overnight. After overnight incubation, membranes were
washed twice in 2X SSC, 0.1% SDS for 15 minutes at room temperature, and then
washed twice in 0.2X SSC, 0.1 % SDS at 55°C for 1 hour. The filters were
autoradiographed at -70°C with Kodak XRR film.

Ethidium bromide-stained agarose gel and Southern hybridization analysis of PCR amplified products from oocyst-seeded fecal samples was performed in order to determine whether the dipstick method described herein resulted in a reduction of inhibition of PCR amplification of *T. gondii*-specific DNA in fecal slurries as compared with fecal slurries alone. Two sets of solutions, PBS and PBS/Feces (1:4 gm/ml), were seeded with four concentrations of oocysts, 2 x 10⁶, 5 x 10⁵, 5 x 10⁴, and 5 x 10³. Using the dipstick technique described above, this resulted in an estimated maximum number of oocysts in the PCR amplification tube to be 400, 100, 10, and 1 as indicated for the PBS solution and for the PBS/Feces solution respectively. Southern hybridization was performed using the OC-2 gene internal primer as the probe. Southern hybridization results and the ethidium bromide stained gel demonstrated that inhibition of PCR amplification of the exogenously added DNA was dramatically reduced (as compared with fecal extract alone) in samples prepared as per the dipstick assay as described above.

Three different paper supports were tested for their ability to support the PCR dipstick assay: IsoCodeJ™ Stix, S&S® #903™ (available from Schleicher and Schuell)

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and Nobuto Blood Filter Strips (available from Advantec, Pleasantville, CA). First, IsoCode JTM Stix were tested for the ability to bind oocysts. Oocysts were diluted into either PBS or a suspension of uninfected feces and PBS. The fecal dipstick procedure as described above was used to sample and elute DNA for PCR analysis. The concentration of oocysts per reaction was adjusted so that theoretical maximum could be 1, 10, 100, and 400 oocysts respectively. The amplification products were run on an agarose gel and stained as described above. According to this assay, oocysts diluted into PBS alone could be readily detected at 10 oocysts per ul of dipstick eluate with primers directed to the *T. gondii* OC-2 gene. In addition, oocysts in a suspension of feces and PBS could be detected when present at a concentration of between 10 and 100 oocysts per ul. This experiment demonstrates that the oocysts are bound to the IsoCode JTM Stix in the presence of feces, are eluted by heat, and following a wash and heat elution step are sufficiently free from inhibitors to be detected by PCR amplification.

Under these conditions, detecting 10 oocysts per ul of eluate from the IsoCodeJ™ Stix is equivalent to detecting oocysts at a concentration of 2.5 x 10⁵ 15 oocysts/gram of feces. Several parameters were tested for their ability to increase the sensitivity of this test. First, two additional paper supports, S&S® #903™ and Nobuto Blood Filter Strips, were tested for both the ability to bind oocysts in the presence of solubilized feces, and the ability to support subsequent PCR detection of oocyst DNA. Each of these filter papers bound T. gondii oocysts, and subsequent PCR amplification 20 with OC-2 primers detected the presence of T. gondii DNA. However, the sensitivity of detection for each of these papers was somewhat less than the sensitivity of the assay when using IsoCodeJ Stix[™]. All three paper supports were also tested for binding of oocysts in the presence of feces over a range of pH from 4 to 9. The S&S® #903™ and Nobuto Blood Filter Strips were most effective at pH 7. Binding of oocysts to the 25 IsoCodeJ Stix[™] was significantly increased at pH 9. All subsequent assays described below used IsoCodeJ Stix[™] and pH 9 for binding of oocysts to dipsticks.

Another approach to increasing the sensitivity of the assay was to use primers from the B1 gene during the PCR amplification reaction. The B1 gene is a multicopy gene that is present at approximately 35 copies per T. gondii genome. Using a B1-

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specific primer resulted in a ten-fold increase in sensitivity, and produced an assay in which 1 oocyst/ul could routinely be detected. This level of sensitivity of the assay correlated with the ability to detect approximately 1 x 10⁴ oocysts/gram of feces.

The sensitivity and specificity of the PCR detection method was tested in experimentally infected animals using flotation and visualization of oocysts as the standard for quantification of oocysts. SPF cats were infected with mouse brain-derived tissue cysts and feces were collected from the cats for twenty-one days. Each sample was analyzed by both direct visualization and the dipstick PCR technique. Following gel electrophoresis of the products from PCR amplification, the results were scored as either positive or negative depending on the presence or absence of the correct genespecific PCR product. Table 12 shows the results of PCR detection using both the *B1* and OC-2 DNA primers for each individual fecal sample. The positive and negative predicative values were 93.2% and 97.2% respectively using the *B1* gene DNA primers and 80.2% and 95.8% respectively using the OC-2 DNA primers.

15 TABLE 12. Sensitivity, specificity and predicative values for the PCR detection of oocysts in experimentally infected cat feces.

Method	Total Samples +/-	f/n ^a	f/p ^b	Sensitivity %	Specificity %	Predictive Value % +/-
Microscopy	69/176	0	0	100	100	100/100
PCR						
B1 Primers	64/171	5	5	94.7	96.7	93.2/97.2
OC-2 Primers	61/161	7	16	89.7	96.4	80.2/95.8

^a false negative

Example 13:

A PCR ELISA was developed for the detection and quantification of PCR amplification products from the PCR dipstick method. In general, digoxigenin-labeled amplified product produced by the PCR dipstick detection method were detected by hybridization to an internal biotinylated B1 gene primer bound to microtiter wells. The

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b false positive

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concentration of PCR labeled digoxigenin fragment was determined using an alkaline phosphatase-linked anti-digoxigenin antibody (available from Boehringer Mannheim Biochemica Gmbh). The alkaline phosphatase activity level was then determined using a standard ELISA reader. This quantitative PCR ELISA method detected oocysts at a lower limit of 1 x 10⁴ oocysts/gram when tested with uninfected cat feces seeded with known concentrations of *T. gondii* oocysts. The method is described in detail as follows.

PCR amplification using B1 gene-specific primers was performed on eluates from the fecal dipstick method herein described. Amplification products were labeled by incorporation of digoxigenin-11-dUTP (DIG-11-dUTP) present in the reaction mix at 2.5 uM. The concentration of dTTP in this reaction mix was reduced to 22.5 uM. The resulting labeled fragment was detected using reagents from the PCR ELISA (DIG Detection) kit (available from Boehringer Mannheim Biochemica Gmbh, Mannheim, Germany). The procedure was as follows. Four ul of the primary amplification reaction product was added to 16 ul of denaturation buffer and incubated at room temperature for 10 minutes. This was mixed with 200 ul hybridization buffer that contained 20 pmol/ml of the biotinylated B1 gene probe. One-half of the hybridization reaction mixture was transferred to a well in a streptavidin-coated microtiter plate and incubated at 50°C for 3 hours with shaking. The plate was washed with washing buffer five times at room temperature and incubated with 100 ul of anti-digoxigenin Fab conjugated with peroxidase at 37°C for 45 minutes. Following five washes, 100 ul of ABTS substrate solution (available from Boehringer Mannheim Biochemica) was added to each well and the color was developed at room temperature for 45 minutes. The optical densities (OD) at 405 nm were read in a spectrophotometer (SpectraMAX 250, available from Molecular Devices Inc., Sunnyvale, CA) and analyzed with Soft Max Pro™ software (available from Molecular Devices Inc.).

Quantification of oocysts in feces by the PCR ELISA technique was compared with quantification by the microscopic analysis. Individual feces from six different cats were collected (as available) at various days post infection. Oocysts were then quantified for each sample by two separate techniques, microscopy and PCR ELISA.

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The results from each of these two methods were in good agreement. Standard regression analysis produced a correlation coefficient of 0.91.

Example 14.

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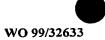
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This example describes the detection of Cryptosporidium parvum oocysts and Giardia lamblia cysts in feces using the PCR dipstick detection method described above. Oocysts and cysts from C. parvum and G. lamblia respectively were detected by the dipstick PCR detection method, thereby demonstrating the usefulness of this method for the detection of cysts or oocysts from unrelated species.

Feline fecal samples from SPF cats were seeded with either C. parvum oocysts or G. lambia cysts and used in the PCR detection method described herein. The primers used to detect C parvum were specific for the C. parvum AWA gene, while the primers used to detect G lambia were specific for the G. lambia ABB gene (Rochelle, et al., 1997, Applied and Environmental Microbiology 63:106-114).

In order to demonstrate binding of *C. parvum* oocysts to a dipstick in the presence of feline fecal slurry, aliquots of feline fecal slurry (1:4, mg/ml) were seeded with between 5X 10² and 5X 10⁶ *C. parvum* oocysts/ml. These samples were then tested for binding of the oocysts and subsequent PCR analysis according to the PCR detection methods described herein. The primers used in the PCR amplification were specific for the *C. parvum* 1WA gene. The PCR amplified products were run on and agarose gel and stained with ethidium bromide. The *C. parvum*-specific primer primed amplification of a DNA prostact of the predicted mobility, in an oocyst concentration-dependent manner, from the depart of the predicted mobility, in the results of this experiment demonstrated that *C. parvum* oocysts bound to a dipstick in the presence of feline fecal slurry, and that about 5X 10⁻¹ (parvum oocysts/ml were detectable by the PCR detection method after binding to the dipstick under these conditions. Because 5X 10² oocysts/ml was the lowest concentration tested, and the products were easily observable, the concentration of cysts detectable by this method is likely to be lower than 5X 10² oocysts/ml.

In order to demonstrate binding of *Giardia* cysts to a dipstick in the presence of feline fecal slurry, aliquots of feline fecal slurry (1:4, mg/ml) were seeded with between 5×10^2 and 5×10^5 G. lamblia cysts/ml. These samples were then tested for binding of



the cysts and subsequent PCR analysis according to the PCR detection methods described herein. The primers used in PCR amplification were specific for the G. lamblia ABB gene. The PCR amplified products were run on and agarose gel and stained with ethidium bromide. The G. lamblia-specific primer primed amplification of a DNA product of the predicted mobility, in a cyst concentration-dependent manner, from the dipstick eluate as described above. The results of this experiment demonstrated that G. lamblia cysts bound to a dipstick in the presence of feline fecal slurry, and that about $5 \times 10^2 \ G$. lamblia cysts/ml were detectable by the PCR detection method after binding to the dipstick under these conditions. Because $5 \times 10^2 \ \text{cysts/ml}$ was the lowest concentration tested, and the products were easily observable, the concentration of cysts detectable by this method is likely to be lower than $5 \times 10^2 \ \text{cysts/ml}$.

Example 15:

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This Example discloses a method of isolation of *T. gondii* nucleic acid molecules encoding immunogenic *T. gondii* proteins recognized by intestinal secretions from infected cats. This Example further discloses recombinant nucleic acid molecules and proteins of the present invention.

The production of intestinal secretions and from infected cats and the use of these secretions for screening for nucleic acid molecules encoding immunogenic *T. gondii* proteins are described herein in Example 6. Intestinal secretions collected from a single cat that had been previously infected with *T. gondii* were pooled and preabsorbed to remove antibodies directed against UCG and *E. coli*. The pooled, preabsorbed intestinal secretions are also referred to herein as MGIS antiserum. MGIS antiserum was used to immune screen an ICG cDNA library in order to identify and isolate nucleic acid molecules encoding immunogenic *T. gondii* proteins recognized by intestinal secretions from infected cats. Six nucleic acid molecules encoding immunogenic *T. gondii* proteins recognized by intestinal secretions from infected cats were identified and isolated using the following methods. These six nucleic acid molecules are referred to herein as MGIS4-2 (also herein referred to as SEQ ID NO:282 and SEQ ID NO:284, representing the coding strand and its reverse complement, respectively), MGIS4-4 (also herein referred to as SEQ ID NO:292 and SEQ ID NO:294), MGIS4-8 (also herein

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referred to as SEQ ID NO:306 and SEQ ID NO:308), MGIS6-5 (also herein referred to as SEQ ID NO:311 and SEQ ID NO:313), MGIS6-2 (also herein referred to as SEQ ID NO:326 and SEQ ID NO:328), and MGIS1-3 (also herein referred to as SEQ ID NO:329 and SEQ ID NO:331).

Absorption of MGIS Antibody

MGIS antiserum was collected, as previously described, from the cat intestine on weeks 6, 10, and 13 after infection, and on weeks 0, 1, 2, 3, 4, and 5 after challenge. Both pools of antisera were combined and used to screen the cDNA library, and are herein referred to as MGIS antiserum.

To remove anti-cat intestinal and anti-*E.coli* tissue reactive antibodies, the MGIS pools were absorbed to nitrocellulose (NC) filters coated with either cat intestinal proteins or *E.coli* proteins. Cat intestinal proteins used to coat the nitrocellulose filters were generated as follow. The epithelial layer of uninfected cat intestine was scraped on dry ice and the cells subsequently passed through several different gauge needles (No. 18, 21, and 23) 10 times each. The sample was frozen and thawed 3 times, and then sonicated on ice for 10 minutes. The protein extract was diluted to 400ug/ml in PBS and immersed with the nitrocellulose at room temperature for 1 hour, and was then blocked with 4% milk in PBS for 30 minutes. Similarly, XL-1 blue *E.coli* cells were resuspended in PBS and bacterial protein extracts prepared similar to the cat intestinal proteins. The bacterial extract was diluted to a final concentration of 2.3 mg/ml in PBS and bound to the filter in a manner similar as the cat intestinal extract.

MGIS antiserum was diluted 1:20 with 4% milk in PBS and absorbed sequentially to both the cat intestinal and bacterial protein coated filters at room temperature for 1 hour. To demonstrate that all UCG and *E. coli*-reactive antibody had been removed from the MGIS antiserum preparation, the MGIS antiserum subjected to Western blot analysis which showed that the absorbed antibody had no reactivity to either the cat intestinal proteins or to the bacterial extract.

Immune Screening of T. gondii cDNA Phage Library

The ICG cDNA library was constructed from infected cat intestinal mRNA, and the cDNA product cloned into the *EcoRI/XhoI* sites of the Uni-Zap XR vector.

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Toxoplasma-specific nucleic acid molecules represented approximately 10% of the library. The ICG cDNA phage library was plated to approximately 2-5x10^{e4-5} pfu per 135 mm plates with XL-1Blue MRF' cells (available from Stratagene). Ten plates were treated in the following manner after the phage were pinhead in size. Nitrocellulose filters that had been previously treated with IPTG were overlaid on top of the phage and incubated at 3°C for 5 hours. The filters were marked, washed with TBS, pH 8.0, blocked with 4° milk in TBS, and incubated with MGIS antiserum at room temperature overnight. After washing three times with TBS, horse-radish peroxidase (HRP)-labeled goat anti-cut lg A antibody (Bethyl Lab. Inc.) was diluted 1:350, and incubated with the filters at room temperature for 2 hours. The color indicator was developed with 4-chloro-1-naphthol substrate and H₂O₂. Forty-one positive clones were selected for further screening

Hybrid: atton Screening and Clone Purification

PCR amplified in a final volume of 12.5 ul containing 1ul of template DNA, 50mM KCL, 10mM 1 ms-HCL (pH 8.3), 2mM MgCl₂, 0.2mM each dNTP, 0.2mM each of T3 and T7 vector specific oligonucleotide primers, and 0.3 units of Taq polymerase.

Amplification was performed by 1 cycle of 95° C for 3 min., 35 cycles of 95° C for 30 sec., 51° (10° 30 sec., and 72° C for 2 min., followed by 75° C for 5 min. on a Perkin Elmer 30° C to 2 min., followed by 75° C for 5 min. on a Perkin Elmer 30° C to 2 DNA transferred to a nylon membrane.

Megaprims DNA labeling systems (available from Amersham International) and used as a probe to analyse the PCR amplified DNA fragments on the nylon membrane. The membrane was pre-hybridized in 5xSSPE (1x SSPE: 0.18M NaCl, 10mM NaH₂PO₄, and 1mM 1 D 1 V pH 7.7), 0.5% SDS, 5x Denhardt's solution, and 0.1mg/ml single stranded salmon sperm DNA at 65° C for 3 hours. Membranes were then hybridized overnight at 65° C, and then washed with 2xSSPE, 0.1% SDS at room temperature for 10 min., twice, and 0.2xSSPE, 0.5% SDS at 65° C for 1 hour, twice. The membrane was exposed to

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film at -70° C overnight. Twenty-three clones were thus shown to contain *T. gondii*-specific DNA, with an insert size of 1-2 Kb in length.

Clone Identification by Phage Drop Test

Each of the twenty-three *T. gondii*-specific clones were rescreened to confirm reactivity with MGIS antiserum. Phage clones were diluted 1:10e7 from the SM buffer stock, and 3ul of this dilution (~5-50 phage) was spotted onto a NZYM/XL-1Blue MRF' agar plate, and incubated at 37° C for 5 hours. Afterwards, an IPTG pre-treated nitrocellulose filter was overlaid onto the agar surface and incubated for another 5 hours. The filter was marked, washed with TBS buffer (pH 8.0) at room temperature for 15 minutes, and blocked with 4% milk in PBS for 30 minutes. Pre-absorbed MGIS antiserum was added to the filter and allowed to react at room temperature overnight. The filter was subsequently washed in TBS at room temperature for 10 minutes, three times. Goat anti-cat IgA polyclonal antibody labeled with HRP (available from Bethyl Laboratories, Inc.) was diluted 1:300 in TBS buffer and incubated with the filter at room temperature for 2 hours. The filter was washed and developed using 4-chloro-1-naphthol substrate and H₂O₂. Thirteen of the 23 clones were identified as positive for expressing antigen recognized by IgA in the MGIS antiserum.

DNA Sequencing

The DNA inserts in the thirteen clones identified as positive were subcloned into
the TA vector using the TA cloning kit (available from Invitrogen). Individual clones
were PCR amplified using the T3 and T7 vector-specific primers. The DNA fragments
produced by PCR amplification were gel electrophoresed on a 1% agarose gel, and gel
purified using a Qiagen Gel Purification kit (available from Qiagen). Plasmid DNA was
purified using the 5 prime 3 prime Perfect Plasmid DNA Preparation kit (available from
5 Prime 3 Prime Inc., Boulder, CO). DNA sequencing was carried out on six of the T.
gondii-specific DNA inserts using a Prizm dideoxy termination kit (available from
Perkin Elmer) on an ABI 377 DNA sequencer (available from Applied Biosystems). TA
sense and TA antisense oligonucleotide primers were used for DNA sequencing, and
insert-specific oligonucleotide primers were used to generate internal fragment
sequences. The only variation from this general protocol was in the case of MGIS4-4,

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where the Erase a Base system (available from Promega) was used to generate plasmids containing deleted fragments in order to facilitate sequencing. The primers used for sequencing each of the inserts were the following:

The primers used in sequencing MGIS4-2 are herein referred to as SEO ID 5 NO:275, SEQ ID NO:276, SEQ ID NO:277, SEQ ID NO:278, SEQ ID NO:279, SEQ ID NO:280, and SEQ ID NO:281. The primers used in sequencing MGIS4-4 are herein referred to as SEQ ID NO:285, SEQ ID NO:286, SEQ ID NO:287, SEQ ID NO:288, SEQ ID NO:289, SEQ ID NO:290, and SEQ ID NO:291. The primers used in sequencing MGIS4-8 are herein referred to as SEQ ID NO:295, SEQ ID NO:296, SEQ 10 ID NO:297, SEQ ID NO:298, SEQ ID NO:209, SEQ ID NO:300, SEQ ID NO:301, SEQ ID NO:302, SEQ ID NO:303, SEQ ID NO:304, and SEQ ID NO:305. The primers used in sequencing MGIS6-5 are herein referred to as SEQ ID NO:309 and SEQ ID NO:310. The primers used in sequencing MGIS6-2 are herein referred to as SEQ ID NO:314, SEQ ID NO:315, SEQ ID NO:316, SEQ ID NO:317, SEQ ID NO:318, SEQ ID NO:319, SEO ID NO:320, SEQ ID NO:321, SEQ ID NO:322, SEQ ID NO:323, SEQ ID NO:324, 15 and SEQ ID NO:325. And the primers used in sequencing MGIS1-3 are herein referred to as SEQ ID NO:314, SEQ ID NO:315, SEQ ID NO:316, SEQ ID NO:317, SEO ID NO:318, SEQ ID NO:319, SEQ ID NO:320, SEQ ID NO:321, SEQ ID NO:322, SEQ ID NO:323, SEQ ID NO:324, and SEQ ID NO:325 (note that the same primers were used 20 for sequencing MGIS6-2 and MGIS1-3).

PCR Amplification of Feline and T. gondii DNA With Clone-specific Primers
The IgA selected MGIS clones were shown to be Toxoplasma specific by PCR
amplification analysis. The following different cDNA samples were tested for the
presence of DNA representing each of the six different IgA-selected nucleic acid
molecules: a) uninfected cat gut (UCG); b) infected cat gut (ICG); c) T. gondii
tachyzoite (TgTz); d) Toxoplasma bradyzoite (TgBz); and e) Toxoplasma genomic
DNA (TgTz DNA). The preparation of UCG, ICG, Toxoplasma tachyzoite and
bradyzoite cDNA was as described above. Toxoplasma genomic DNA was isolated
from tachyzoites by phenol/chloroform/isoamylalcohol pH 8.0 extraction.

Oligonucleotide sense and anti-sense primers specific to each of five MGIS-selected nucleic acid molecules were synthesized and used as primers in the PCR amplification reactions. The reaction condition were: 95 °C for 10 min., followed by 35 cycles of 95 °C for 30 sec., 58 °C for 30 sec., 72 °C for 40 sec; this was followed by 75 °C for 5 min. afterwards to complete the reaction. The amount of the different templates used in the PCR reactions (~3-30 ng of DNA), was empirically determined by comparison with a PCR amplified *Toxoplasma* tubulin gene product standard generated with each template. The oligonucleotide primers and the size of the expected products are listed in Table 13, below.

10 Table 13.

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MGIS Clone	Sense Primer Position: Sequence	Anti-Sense Primer Position: Sequence	Product Size (bp)
1-3	1513: SEQ ID NO: 319	1858: SEQ ID NO: 320	346
4-2	168: SEQ ID NO: 276	594: SEQ ID NO: 279	427
4-4	455: SEQ ID NO: 285	775: SEQ ID NO: 290	331
4-8	2018: SEQ ID NO: 300	2310: SEQ ID NO: 301	293
6-2	1301: SEQ ID NO: 319	1646: SEQ ID NO: 320	346

The oligonucleotide primers specific for each of the five MGIS-selected nucleic acid molecules PCR amplified products only when the template DNA contained *Toxoplasma* DNA. There were no PCR amplified products in this assay when the template DNA was UCG cDNA. These results confirm the *T. gondii* origin of the MGIS-selected nucleic acid molecules.

Sequence Analysis

Homology searches of a non-redundant protein database were performed on all six MGIS-selected nucleic acid molecules, translated into all six reading frames, using the BLASTX program available through the BLASTTM network of the National Center for Biotechnology Information (NCBI) (National Library of Medicine, National Institute of Health, Baltimore, MD). This database includes SwissProt + PIR + SPupdate + GPUpdate + PDB databases. In addition, BLASTN homology searches were

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performed on these sequences using the NCBI databases including the non-redundant database of GenBank EST, and genembl. In all cases, the default parameters for the homology programs were used.

The highest scoring match of the homology search (BLASTX) of translation products of the nucleic acid sequence SEQ ID NO:282 (MGIS4-2) was to GenBank[™] Accession No. prf 2208369A, a *Homo sapiens* signal peptidase 12kD subunit protein. The protein encoded by nucleic acid residues 742-945 of MGIS4-2 (SEQ ID NO:282) showed about 44% identity to amino acid residues 12 to 79 of the protein represented by GenBank[™] Accession No. prf 2208369A. At the nucleotide level, SEQ ID NO:282 showed 97% identity over 353 nt with te sequence represented by GenBank[™] Accession No. W0680 (TgESTzy81e12.r1), an EST fragment isolated from *T. gondii* tachyzoite cDNA. The homology spans the region from nt 748 to nt 1097 of SEQ ID NO:282, and nt 15 to 365 of GenBank[™] Accession No. W0680. There were no other significant homology matches to SEQ ID NO:282 nucleic acid sequence.

The highest scoring matches of the homology search (BLASTX) of translation products of the nucleic acid sequence SEQ ID NO:292 (MGIS4-4) were to proteins described as elongation factor 1-gamma, with the highest match to the sequence represented by GenBank™ Accession No. gi 2160158, described as "a protein similar to elongation factor" The protein encoded by residues 47-1222 of SEQ ID NO:292 showed about 37% identity to amino acid residues 5-414 of the protein represented by GenBank™ Accession No. gi 2160158. At the nucleotide level SEQ ID NO:292 showed 94% identity over 413 nt with an EST fragment, GenBank™ Accession No. N81326 (TgESTzy40a12.r1), an EST fragment isolated from *T. gondii* cDNA. The homology spans the region from nt 420 to nt 832 of SEQ ID NO:292, and nt 15 to 427 of GenBank™ Accession No.N81326. In addition, SEQ ID NO:292 showed 99% identity over 187 nt with an EST fragment, GenBank™ Accession No. W05869 (TgESTzy85a09.r1), an EST fragment isolated from *T. gondii* cDNA clone. The homology spans the region from nt 757 to nt 943 of SEQ ID NO:292, and nt 62 to 248 of GenBank™ Accession No.W05869.

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The highest scoring match of the homology search (BLASTX in the genembl database) of translation products of the nucleic acid sequence SEQ ID NO:329 (MGIS1-3) was to Herpesvirus Saimiri complete genome, represented by GenBank[™] Accession No. X64346. The amino acid residues 777 to1432 of the protein encoded by reading frame +2 of SEQ ID NO:329 showed about 36% identity to amino acid residues 106974 to 106517 of the protein represented by GenBank[™] Accession No. X64346. At the nucleotide level, SEQ ID NO:329 showed 94% identity over 578 nt with an EST fragment, GenBank[™] Accession No. AA520348 (TgESTzz69d04.r1), an EST fragment isolated from *T. gondii* bradyzoite cDNA. The homology spans the region from nt 1334 to1910 of SEQ ID NO:329, and nt 5 to 571 of GenBank[™] Accession No. AA520348.

The highest scoring match of the homology search (BLASTN of the non-redundant databases, GenBank+EMBL+DDBJ+PDB) of SEQ ID NO:311 (MGIS6-5) was to a *T. gondii* lactate dehydrogenase gene, represented by GenBank[™] Accession No. TGU35118. SEQ ID NO:311 showed 99% identity over 1619 nt.

The highest scoring match of the homology search (BLASTX in the genembl database) of translation products of the nucleic acid sequence SEQ ID NO:326 (MGIS6-2) was to Herpesvirus Saimiri complete genome, represented by GenBank[™] Accession No. X64346. Amino acid residues 751 to1206 encoded by SEQ ID NO:326 showed about 36% identity to amino acid residues 106972 to 106517 of the protein represented by GenBank[™] Accession No. X64346. At the nucleotide level, SEQ ID NO:326 showed 96% identity over 247 nucleotides with an EST fragment, GenBank[™] Accession No. AA520348 (TgESTzz69d04.r1), an EST fragment isolated from *T. gondii* bradyzoite cDNA. The homology spans the region from nt 890 to1136 of SEQ ID NO:326, and nt 144 to 390 of GenBank[™] Accession No. AA520348.

The highest scoring match of the homology search (BLASTX of the non-redundant GenBank CDS database including

Translations+PDB+SwissProt+SPupdate+PIR) of translation products of the nucleic acid sequence SEQ ID NO:306 (MGIS4-8) was to a rice 26S protease regulatory subunit 4 homolog (TAT-binding protein homolog 2), represented by GenBankTM Accession No.

P46466. 26S protease regulatory subunit 4 homologs representing other species also

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have high homology to a translation product of SEQ ID NO:306. The protein encoded by nucleic acid residues 465 to 1565 of SEQ ID NO:306 showed about 72% identity to amino acid residues 35 to 448 of the protein represented by GenBank™ Accession No. X64346. It should be noted a gap of 42 amino acids was required in the amino acid sequence encoded by SEQ ID NO:306 in order to achieve the sequence fit resulting in this high homology. At the nucleotide level, SEQ ID NO:306 showed 98% identity over 269 nucleotides with an EST fragment, GenBank™ Accession No. W35531 (TgESTzy90g01.r1), an EST fragment isolated from *T. gondii* cDNA. The homology spans the region from nt 668 to nt 936 of SEQ ID NO:326, and nt 23 to nt 291 of GenBank™ Accession No. W35531.

Example 16:

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This Example discloses the isolation and sequence analysis of a 1397 bp T. gondii nucleic acid molecule composed of four fragments isolated by subtractive selection from an infected cat gut cDNA library. Also described is an additional nucleic acid molecule representing the genomic DNA sequence immediately upstream (5') of, and overlapping, the genomic DNA sequence encoding the cDNA sequence.

A 1397 bp *T. gondii* nucleic acid molecule, denoted nTG₁₃₉₇ (the coding strand of which is herein referred to as SEQ ID NO:343, and the reverse complement of which is herein referred to as SEQ ID NO:345), is a composite of four overlapping PCR amplified products isolated from an infected cat gut (ICG) cDNA library. Specifically, a first 424 bp fragment (representing nucleotide positions 709-1132 of SEQ ID NO:343), was isolated after two rounds of selection using the PCR-Select™ Subtraction kit (available from Clontech, Palo Alto, CA), using day eight, *Rsa*I restriction enzyme digested ICG cDNA as tester, and similarly digested uninfected cat gut cDNA as driver DNA. Fragments enriched by the PCR-Select™ Subtraction selection process were digested with the restriction enzyme *Sma*I and cloned into *Sma*I site in the commercially available positive selection vector, QuanTox™ (available from Quantum Biotechnologies Inc., Laval, Quebec, Canada). The cloned inserts were subsequently sequenced using the oligonucleotide primers, T7 (TAATACGACTCACTATAGGG, herein referred to as SEQ ID NO:348) and T3 (ATTAACCCTCACTAAAGGGA, herein

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referred to as SEQ ID NO:347). A 424 bp T. gondii nucleic acid molecule, referred to herein as nTG_{424} , was isolated, cloned and sequenced by this method.

The orientation of nTG₄₂₄, as well as additional nucleic acid sequence representing cDNA sequence occurring downstream (3') of nTG₄₂₄ was determined as 5 follows. A 689 bp fragment including the 3'-end of the gene comprising nTG₄₂₄ was generated by PCR amplification of an ICG cDNA library constructed in the Uni-Zap XR insertion vector (available from Stratagene). The two primers used for this amplification reaction are represented by SEQ ID NO:358 (709ACAACGACCACGACATCAACTAC731, derived from the sequence of nTG424, also 10 referred to as pRay8), and an adaptor oligonucleotide primer that hybridized to the cDNA poly A tail (GGCCACGCGTCGACTACT₁₇ from BRL/GIBCO, Gaithersburg, MD, herein referred to as SEQ ID NO:364). The superscript numbers at the beginning and end of the primer sequences described herein represent the location of the primer sequence relative to nTG₁₃₉₇ (SEQ ID NO:343). A resulting 689bp T. gondii nucleic acid 15 molecule (also referred to as nTG₆₈₉) was cloned into PCR2.1 (available from Invitrogen, Carlsbad, CA), and sequenced using the M13 reverse oligonucleotide primers (CAGGAAACAGCTATGACC, herein referred to as SEQ ID NO:346) and the T7 oligonucleotide primer (SEQ ID NO:348). The sequence of nTG₆₈₉ revealed 266 bp of additional cDNA sequence (from 1133-1397 bp, relative to SEQ ID NO:343), with an 20 overlap with nTG₄₂₄ from 709-1132 bp (relative to SEQ ID NO:343). There were three nucleotide differences between the sequence data for nTG424 and the sequence data for nTG₆₈₉. Instead of a "T", "C" and "T" nucleotide at positions 1159, 1166, and 1169 respectively, the sequence data for nTG₆₈₉ revealed a "C", "T", and "A" at those positions.

The remainder of the nucleic acid sequence of nTG₁₃₉₇ was determined in two PCR amplification steps using the ICG cDNA library as the template. The primers for the first PCR amplification were: a) an anti-sense oligonucleotide primer specific for nTG₄₂₄, having the sequence ⁹²⁹GTTGTCGTAGATGTCGTTGTAGTT⁹⁰⁶, and herein referred to as SEQ ID NO:359; and b) a Uni-Zap XR insertion vector-specific oligonucleotide primer (available from Stratagene, and referred to as Tp277) having the

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sequence, GGGAACAAAAGCTGGAGCTCCACC, and herein referred to as SEQ ID NO:354. In the first PCR amplification step, SEQ ID NO:359 and SEQ ID NO:354 were used to generate an 884 bp nucleic acid molecule, (825 bp of which was nTG₁₃₉₇-specific DNA sequence), that was then cloned into PCR2.1. The *T. gondii*-specific nucleic acid molecule is herein referred to as nTG₈₂₅. nTG₈₂₅ was sequenced using a TA sense oligonucleotide primer (having the sequence, CGAGCTCGGATCCACTAG, herein referred to as SEQ ID NO:350), and a TA anti-sense oligonucleotide primer (having the sequence, GCCAGTGTGATGGATATCTGCAG, herein referred to as SEQ ID NO:349), as well as a nTG₁₃₉₇-specific internal oligonucleotide primer having the sequence, ⁵⁶⁴GAGGAGATCGAACTTTGCTTGTGC⁵⁴¹, herein referred to as SEQ ID NO:361. Sequencing revealed that nTG₈₂₅ added an additional 604 bp to the sequence of nTG₁₃₉₇, from nucleotides 105-708 (relative to SEQ ID NO:343). nTG₈₂₅ overlapped with nTG₄₂₄ and nTG₆₈₉ from base 709-939 (relative to SEQ ID NO:343).

The primers for the second PCR amplification step were: a) an oligonucleotide primer specific for nTG₄₂₄, having the sequence

²²⁵AGAAGCGCCTTTGCGTTTCTACGT²⁰², herein referred to as SEQ ID NO:360; and b) Tp277. These two primers were used to generate a 225 bp *T. gondii* DNA fragment, referred to as nTG₂₂₅. nTG₂₂₅ cloned into PCR2.1, and nucleotide sequenced with the TA oligonucleotide primers as above, thereby generating the sequence from nucleotides 1-104 of SEQ ID NO:343. Sequence analysis revealed that nTG₂₂₅ overlapped with previously isolated nTG₈₂₅ DNA sequence from base105-225, relative to SEQ ID NO:343.

The contiguous cDNA sequence of the overlapping fragments representing nTG₁₃₉₇ was determined (and referred to herein as SEQ ID NO:343), and sequence analysis of the composite molecule revealed an 867 bp coding region (referred to as nTG₈₆₇), assuming an initiation codon at position 238-240, and a stop codon at position 1102-1104 (relative to SEQ ID NO: 343). The coding strand of nTG₈₆₇ is herein referred to as SEQ ID NO:340, and the reverse complement is herein referred to as SEQ ID NO:340. Translation of the coding region of nTG₈₆₇ yields a 288 amino acid protein

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herein referred to as PTg₂₈₈, the amino acid sequence of which is herein referred to as SEQ ID NO:341.

To confirm the DNA sequence in the predicted coding region of nTG₁₃₉₇, a PCR amplified fragment containing nucleotides 238 to 1271 was generated using an oligonucleotide primer having the sequence, AAGGATAGGCGGCCGCAGGTACC 5 ²³⁸ATGGCAGGAAGGCAGGCGTT²⁶⁰, herein referred to as SEQ ID NO:362, and an oligonucleotide primer having the sequence, ACCGCTCGAGAAGCTT ¹²⁷¹GAAGCCAAGACATCCCTTCGTGCA¹²⁴⁸, herein referred to as SEQ ID NO:363. The nucleotides in italics represent non-nTG₁₃₉₇ nucleotide sequence, and were present to attach convenient restriction sites to the PCR product. The resulting PCR fragment was 10 cloned into a eukaryotic expression vector, referred to as pDVacIII, and sequenced using two vector-specific oligonucleotide primers: a) Tp244, having the sequence, GGATGCAATGAAGAGGGGCTC, and herein referred to as SEQ ID NO:352; and b) Tp245, having the sequence, AACTAGAAGGCACAGTCGAGGCTG, and herein referred to as SEQ ID NO:353. The PCR fragment thus generated contained two 15 nucleotide differences as compared with the previously determined cDNA sequence of nTG₁₃₉₇. Instead of an "A" at position 643, a "G" residue was found, and in place of a "T" at position 1187, a "C" residue was found. The resulting nucleotide change at position 643 altered the predicted encoded amino acid from an arginine to a glycine residue. The change at position 1187 did not change the predicted amino acid sequence 20 of nTG₁₃₉₇.

Genomic DNA sequence upstream of the gene comprising nTG₁₃₉₇ was determined by generating a 747 bp fragment by PCR amplification of the λ-EMBL-3 Sau3A partial Toxoplasma genomic library herein described. The primers used were SEQ ID NO:360 (representing nucleotides 202-225 in nTG₁₃₉₇) and a λ-EMBL-3-specific primer having the sequence, GGTTCTCTCCAGAGGTTCATTAC, and herein referred to as SEQ ID NO:351. The resulting DNA fragment was cloned in PCR2.1 and sequenced with TA oligonucleotide primers (SEQ ID NO:349, and SEQ ID NO:350) and two gene specific oligonucleotide primers, Tp310

30 (365CGGACGTTGCATGTCAGTGGACA343, herein referred to as SEQ ID NO:355) and

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Tp311 (²⁴³CACGAAGCTGCATGTTCCAGCTAG²⁶⁵, herein referred to as SEQ ID NO:356). The sequence of the PCR fragment revealed a 647 bp DNA fragment, nTG₆₄₇, (herein referred to as SEQ ID NO:338, the reverse complement is herein referred to as SEQ ID NO:339₃ including 421 nucleotides of new genomic DNA sequence upstream of the 5' end of the cDNA sequence of Tg₁₃₉₇. The fragment contained 327 bp of genomic DNA sequence that overlapped with the cDNA sequence, SEQ ID NO:343 (in other words, bases 422-647 of the genomic DNA sequence, SEQ ID NO:338, overlap with bases 1-225 of the cDNA sequence, SEQ ID NO:343). There was a single nucleotide difference between the genomic and the cDNA sequences at position 118 of the cDNA sequence (SEQ ID NO:343), where there is a "G" in the genomic DNA sequence and an "A" at the equivalent position in the cDNA sequence.

SEQ ID NO:343 was shown to be T. gondii specific by PCR amplification analysis of various DNAs, using nTG₁₃₉₇-specific DNA primers to drive the reaction. The following cDNA samples were tested for the presence of nTG₁₃₉₇ DNA: a) uninfected cat gut (UCG), b) infected cat gut (ICG), c) T. gondii tachyzoite (TgTz), and d) Toxoplasma bradyzoite (TgBz). To generate UCG and ICG RNA, gut tissue samples from an uninfected cat and a cat 7 days post infection with T. gondii tissue cysts (1000 cysts) were processed by scraping and collecting the epithelial layer of gut cells on dry ice. Cells from UCG, ICG, and T. gondii tachyzoites and bradyzoites were solubilized by homogenization in TRI-reagent (available from Molecular Research Center Inc., Cincinnati, OH), and the homogenate passed through a 18/20/and 22 gauge needle 10 times each sequentially. After standing at room temperature for 5 min., 100 ul of bromochloropropane (available from Molecular Research Center Inc.)/ ml of TRI reagent was added, and the homogenate vortexed for 15 seconds. The sample was centrifuged at 14,000 rpm for 15 min. at 4°C, the aqueous layer collected, and RNA precipitated with one half volume of isopropanol. Contaminating genomic DNA was removed by digestion with 10 units of RNase free DNaseI (available from Boehringer Mannheim Corp.) at 37 °C for 30 min. The sample was then extracted with phenol/chloroform/isoamylalcohol, pH 6.0. The RNA was precipitated from the aqueous layer with ethanol and resuspended in diethylpyrocarbonate (available from

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Sigma) treated water. cDNA was generated from total RNA using a commercially available RT-PCR kit (available from Stratagene).

Two nTG₁₃₉₇-specific oligonucleotide primers were used in the reaction: SEQ ID NO:358, having the sequence, ⁷⁰⁹ACAACGACCACGACATCAACTAC⁷³¹, and SEQ ID NO:357, having the sequence, ¹¹¹⁴ACACTTTGGTCTAATCGAGGGTAG¹⁰⁹¹. The reaction conditions were: 95 °C 12 min., followed by 3 cycles of 94 °C 30 sec., 70 °C 30 sec., 72 °C 60 sec., 3 cycles of 94 °C 30 sec., 72 °C 60 sec., 3 cycles of 94 °C 30 sec., 65 °C 30 sec., 72 °C 60 sec., 67 °C 30 sec., 63 °C 30 sec., 72 °C 60 sec., 25 cycles of 94 °C 30 sec., 59 °C 30 sec., 72 °C 60 sec., and a seven minute extension at 75 °C to complete the reaction. The amount of template used in each PCR reaction (~3-30 ng of DNA), was empirically determined by comparison with a PCR amplified *Toxoplasma* tubulin gene product standard generated with each template. The PCR amplification reaction generated a 406 bp product only in the reactions containing tachyzoite and ICG cDNA template DNA, thereby confirming the *T. gondii*-specificity of SEQ ID NO:343.

Sequence Analysis

Homology searches of a non-redundant protein database were performed on SEQ ID NO:340 (representing the coding region of nTG₁₃₉₇, translated in frame 1, using the BLASTP program available through the BLASTTM network of the National Center for Biotechnology Information (NCBI) (National Library of Medicine, National Institute of Health, Baltimore, MD). This database searched was PIR. In addition, a BLASTP homology search was performed on SEQ ID NO:341 (representing the amino acid sequence encoded by SEQ ID NO:340) using the NCBI database SwissProt. In all cases, the default parameters for the homology programs were used. Another homology search was run on SEQ ID NO:343 using the BLASTN search program and the database genembl.

When run against the PIR database, the highest scoring match of the homology search of translation products of the nucleic acid sequence SEQ ID NO:340 (the coding strand of the coding sequence) was to GenBankTM accession number A60095, a *Drosophila* larval glue protein precursor. Other significant homologies included

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homology to an African clawed frog mucin, and a promastigote surface antigen-2. When analyzed by the GCG program, using BESTFIT and default parameters, amino acid residues 145 to 281 of the protein encoded by SEQ ID NO:340 showed about 70% identity to amino acid residues 42 to 178 of the protein represented by GenBankTM accession number A60095. In addition, amino acid residues 153 to 282 of the protein encoded by SFQ ID NO:340 showed about 73% identity to amino acid residues 394 to 523 of the protein represented by GenBankTM accession number A45155 (African clawed frog mucin). When compared with the SwissProt database, the highest scoring match of the homology search of the amino acid sequence SEQ ID NO:341 (the protein encoded by SLQ ID NO:340) was to GenBankTM accession number Q05049, the African clawed troy mucin. These two amino acid sequences showed a 73% identity from amino acid 153 to 252 of SEQ ID NO:341 and amino acid 394 10 523 of the amino acid sequence represented by GenBankTM accession number Q05049. A comparison of SEO ID NO 343 (the cDNA coding strand) using the BLASTN search program and the database general revealed a 76% nucleic acid sequence identity to a D. discoideum protein kinase. GenBankTM accession number M38703. This identity was between nt 765 to 1058 of SI Q ID NO:343 and nt 772 to 1065 of the sequence represented by GenBank Maccession number M38703. In addition, a BLASTN comparison SEO ID NO:343 with the non-redundant GenBankTM database including GenBank EMBI · DDB revealed an 89% identity between nucleic acid residues 779 to 902 of SEQ 11) See 13 and nt 2150 to nt 2273 of the nucleic acid sequence represented by GenBank 1st accession number DDDU86962.

Example 17

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25 proteins expressed by the *T. gondii* nucleic acid molecules of the present invention. Protein immunization with *T. gondii* recombinant protein and several different adjuvants induced both antibodies and T cell proliferative responses in cats. DNA immunization of cats with plasmid constructs expressing *T. gondii* immunogenic proteins of the present invention also induced antibody responses.

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Protein Immunization

Protein immunization of cats was carried out with three primary subcutaneous immunizations at intervals of four weeks (prime at week 0 and boosts at weeks 4 and 8) using 50 µg protein per injection in adjuvant. The primary antigen was OC-22, which was purified as a HIS fusion protein from *E. coli*. The experimental groups were as follows: two cats were immunized with OC-22 protein in alum, two cats were immunized with OC-22 protein in polyphosphazine (PCPP), and two cats were immunized with OC-22 protein in BAYER1005 (Stunkel, K.G., et al., in *Cellular Basis of Immune Modulation*, 1989, pp. 575-579, incorporated herein by reference in its entirety). One cat was injected with two different antigens in BAYER1005: 50 ug of OC-22 and 12 ug of protein 4499-9. One control cat was injected with saline.

Whole blood was collected from all of the animals at intervals before and after the immunizations. Mononuclear cells were selected from the blood for T cell proliferation analysis (see blow) and the remaining plasma processed for detection of humoral responses. The presence of antibody was determined by western blot analysis and by ELISA using recombinant purified antigens. The western blot analysis was more sensitive at detecting a positive or negative response, while the ELISA provided a more quantitative comparison of the cat's responses to the immunogenic proteins.

Western blot analysis was performed on Recombinant purified OC-22 protein was loaded at 2 ug per lane and blotted to nitrocellulose. Samples were from pre-immune cats and cats at 1, 3, and 5 weeks after immunization. Recombinant purified OC-22 protein was loaded at 2 ug per lane and blotted to nitrocellulose. Analysis of the sera collected at three weeks following the first immunization demonstrated that all seven cats responded positively to OC-22 protein. Both anti-cat IgG and anti-cat IgA were used as secondary antibodies (on separate blots). The westerns showed that OC-22 protein elicited both IgA and IgG responses, although the IgA response was not as strong as the IgG response. The ELISA titers were monitored throughout the immunization regimen. The sera collected at week eight and a half, immediately following the second boost had detectable ELISA titers equal to or greater than 1:10,000 for all seven cats. These analyses did not demonstrate any apparent differences between the cats

immunized with different adjuvants. The single cat immunized with 12 ug of 4499-9

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protein was not positive to 4499-9 protein by either western blot analysis or ELISA, although the same cat demonstrated immune responses to OC-22 that were comparable to the other cats in the study.

Cellular responses to the recombinant *T. gondii* OC-22 protein were tested by *in vitro* proliferation of isolated peripheral blood mononuclear cells (PBMC) to purified protein at concentrations ranging from 0.5 to 8 µg /ml. At higher concentrations of protein, non-specific stimulation was evident, making interpretation difficult, but at lower concentrations of antigen, distinct differences were seen between cats. One week after the first boost, T cells from all of the cats in either the PCPP or BAY R1005 adjuvant groups demonstrated stimulation indices (SI) greater than 3. Cells from the PBS control and two alum group cats did not show any proliferative responses. Peak proliferative responses were seen one week after each boost, with the highest responses observed after the first boost. The cats immunized with protein in PCPP had the highest responses, followed by the cats immunized with protein in BAY R1005. The responses observed at 0.5 µg antigen per ml were lower than the responses observed at higher doses, but correlated well with the results observed at 2 ug/ml (data not shown). All of the immunized cats responded to antigen, at some point during the experiment, with an SI level above 3.

DNA Immunization

20 Cats were immunized with the recombinant eukaryotic expression vector, pDVac II, encoding *T. gondii* nucleic acid molecules encoding the immunogenic proteins OC-2, OC-22, and Tg-50. The pDVacII vector contains the CMV promoter and intron A sequences. The protein expressed by this vector includes the *T. gondii* antigen of interest, fused at the 5 prime end to the tissue plasminogen activator signal sequence and fused at the three prime end with both a stretch of poly histidines and an amino acid epitope from the mammalian *myc* gene. Fifteen cats were divided into four experimental groups: three cats received saline (cats 1, 8, and 16), four cats received DNA encoding OC-2 (cats 2, 5, 9, and 15), four cats received DNA encoding OC-22 (cats 3, 6, 10, and 12), and four cats received a combination of DNA encoding OC-2, OC-22, and Tg-50 (cats 4, 7, 13, and 14). Each cat was injected intramuscularly with a total 300 ug of DNA at two sites per immunization. The combined formulation included 300 ug of each

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plasmid per injection. The cats were given one injection and then at eight weeks received a boost.

The serum samples collected at six weeks after the primary immunization were analyzed. Two out of eight cats immunized with OC-2 DNA were shown to sero convert to antibody positive to OC-2 protein by western blot analysis. None of the sera collected at this time from the cats immunized with OC-22 or Tg-50 DNA were positive by western blot analysis to OC-22 or Tg-50 protein respectively. When sera collected one week following the boost (week 9) were analyzed by western blots, seven of eight cats immunized with OC-2 were positive to OC-2, six of eight cats immunized with OC-22 were positive to OC-22, and one of four cats immunized with Tg-50 were positive to Tg-50. Similar to the western blot analysis for the protein immunogenicity study described above, faint IgA responses from all of the OC-22 sero-positive animals could be observed. ELISA analysis of sera taken one week after the boost indicated that four out of eight cats immunized with OC-2 and four of eight cats immunized with OC-22 had midpoint titers greater then 1:1000.

The T cell analysis demonstrated positive proliferative responses to several antigens, however the data were difficult to interpret. Cells isolated from two cats immunized with the OC-22 gene and one cat immunized with the OC-2 gene each demonstrated significant SI responses. However, the same cells from each of these cats were also stimulated by the other recombinant antigen; i.e. cells from OC-22-injected cats responded to OC-2 protein and cells from OC-2-injected cats responded to OC-22 protein. Sera from these animals did not react with the poly histidine or *myc* fusions on other control fusion proteins. This inability to demonstrate strong proliferative responses in PBMC is consistent with other results observed while exploring the induction of proliferative responses in T cells from DNA immunized cats. Cat peripheral blood is a poor source of responsive T cells.

Analysis of Oocyst Shedding in Protein an DNA immunized cats:

Analysis of oocysts shed following tissue cyst challenge of cats in both the protein and DNA immunogenicity studies showed no significant difference in oocyst shedding between any of the test groups and the control within each study. However, the number of animals in these studies varied between two and four per group, and thus

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this result is statistically meaningless. However, significant reduction, i.e., greater than several logs of total oocysts, was not observed in this experiment.

Example 18:

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This example describes immunization of cats with nucleic acid molecules encoding immunogenic *T. gondii* proteins, and subsequent challenge of the immunized cats.

Immunization Protocol:

The following set of conditions were used for the delivery of DNA-coated gold particles to cats: 1.25 ug of DNA was delivered per shot by Gene Gun (available from Biorad). 1.6 micron gold particles were used in the presence of 0.05 mg/ml PVP (polyvinyl pyrrolidine, 360 kD). The micro-carrier loading quantity was 0.5 mg DNA/cartridge, while the DNA loading ratio was 2.5 ug DNA/mg gold. The animals were anesthetized and shaved at the points of contact with the gun. A total of six shots were delivered to the animal for each immunization: three shots to the inner thigh at 300 psi and three shots to the lower side of the abdomen at 600 psi. The immunization regimen consisted of one prime and two boosts at six week intervals. Tissue cyst challenge was performed two weeks following the second boost. The challenge was with 1000 mouse brain-derived tissue cysts.

The plasmid containing the human growth hormone (hGH) gene was used in the control groups and as a marker in the other groups in all studies. In most control groups, the hGH plasmid was diluted to a concentration similar to that in the test groups. Humoral immune responses to the gene product were measured with an ELISA assay, and cellular responses were measured using hGH protein.

First immunogenicity study: The first immunization was followed by a challenge of 1000 mouse brain derived tissue cysts fourteen weeks later. Sample collection was terminated three weeks after that. There were four groups of five animals per group, as follows: Group 1: Control, hGH (0.125 ug/shot), pDVacIII (1.125 ug/shot) This group received one prime and two boosts, at 0, 6 and 12 weeks, respectively. Group 2: OC-22 in pDVacIII (1.25 ug/shot). This group received one prime and two boosts, at 0, 6 and 12 weeks, respectively. Group 3: hGH (0.125 ug/shot), 9 Toxoplasma nucleic acid molecules OC-2, OC-22, OC-13, OC-14, Tg-41, Tg-45, Tg-50, 4604-3, and 4CQA11

(0.125 ug/shot). This group received one prime and two boosts, at 0, 6 and 12 weeks, respectively. Group 4: hGH (0.125 ug/shot), the same DNA as in Group 3 (9 Toxoplasma nucleic acid molecules), but this group received one prime and one boost, at 6 and 12 weeks, respectively. ELISA analysis for hGH sero conversion using sera collected throughout the study demonstrated that five of five cats in Group 1 were positive (i.e., demonstrated an end point titer > 1,000). Three of five animals in Group 3 were sero-positive to hGH. ELISA analysis for sero conversion to OC-22 protein using sera from Group 2 and Group 3 indicated that three of five and zero of five cats were positive respectively. These data suggest that competition from the other plasmids reduced the rate of sero conversion to an individual plasmid. In all cases positive titers did not occur until after the first boost. Specific-T cell proliferative responses using PBMC from animals in each group were not observed. Using the B1 gene-based PCR ELISA herein described, the average number of oocysts shed for each group was: Group 1, 1.03e8; Group 2, 1.11e8; Group 3, 5.79e7 and Group 4, 8.83e7. Statistical analysis of the data indicated no significant difference between the test groups and the control.

Second immunogenicity study:

The first immunization for this study was followed by a challenge of 1000 mouse brain derived tissue cysts fourteen weeks later. Sample collection was terminated three weeks after that. There were four groups of five animals per group, and all animals received one prime and two boosts. Group 2 consisted of DNA representing 18 nucleic acid molecules of the present invention. Group 3 represent 14 additional nucleic acid molecules of the present invention. Group 4 was a combination of both of these groups. The specific nucleic acid molecules and concentrations used in the immunizations were as follows: Group 1: Control, hGH (0.083 ug/shot), pDVacIII (1.125 ug/shot). Group 2: hGH (0.070 ug/shot), 18 Toxoplasma nucleic acid molecules (BZ1-2, 4604-2, 4604-62, 4CQA27, 4CQA29, 4CQA21, 4CQA27, 4604-62, Q2-4, R8050-6,Tg50, M2A1, M2A5, M2A7, M2A11, M2A19, M2A22, M2A29) (0.070 ug/shot). Group 3: hGH (0.083 ug/shot), 14 Toxoplasma nucleic acid molecules (M2A3, M2A21, M2A18, M2A20, M2A24, M2A6, Q2-9, Q2-10, Q2-11, 4604-63, 4604-17, 4604-69, 4604-54,

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4CQA19) (0.083 ug/shot). Group 4: hGH (0.040 ug/shot), 32 Toxoplasma nucleic acid molecules (BZ1-2, 4604-2, 4604-62, 4CQA27, 4CQA29, 4CQA21, 4CQA27, 4604-62, Q2-4, R8050-6,Tg-50, M2A1, M2A5, M2A7, M2A11, M2A19, M2A22, M2A29,M2A3, M2A21, M2A18, M2A20, M2A24, M2A6, Q2-9, Q2-10, Q2-11, 4604-63, 4604-17, 4604-69, 4604-54, 4CQA19) (0.040 ug/shot).

The ELISA analysis of antibody to hGH protein demonstrated that two of five, three of five, zero of five, and two of five animals seroconverted in Groups 1, 2, 3, and 4 respectively. Using low amounts of hGH plasmid in the presence of eighteen or thirty-two additional plasmids containing nucleic acid molecules of the present invention still induced sero conversion in several animals per group. This observation suggests that there is not a strict reduction in the production of antibodies when a gene is injected with several other constructs.

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What is claimed is:

molecule from said library.

- An isolated nucleic acid molecule encoding an immunogenic *T. gondii* protein that can be identified by a method comprising: a) immunoscreening a library selected from the group consisting of a *T. gondii* genomic expression library and a *T. gondii* cDNA expression library with an antiserum, wherein said antiserum is selected from the group consisting of antiserum raised against *T. gondii* oocysts, antiserum raised against *T. gondii* bradyzoites, antiserum raised against *T. gondii* infected cat gut, and antiserum isolated from a cat immune to *T. gondii* infection; and b) identifying a nucleic acid molecule in said library that expresses a protein that selectively binds to an antibody in said antiserum.
- A method to isolate a nucleic acid molecule encoding an immunogenic T. gondii protein, said method comprising: a) immunoscreening a library selected from the group consisting of a T. gondii genomic expression library and a T. gondii cDNA expression library with an antiserum selected the group consisting of antiserum raised against T. gondii oocysts, antiserum raised against T. gondii bradyzoites, antiserum raised against T. gondii infected cat gut, and antiserum from a cat immune to T. gondii infection; b) identifying a nucleic acid molecule in said library that expresses a protein that selectively binds to an antibody in said antiserum; and c) recovering said nucleic acid
- 3. An isolated immunogenic *T. gondii* protein that can be identified by a method comprising: a) immunoscreening a library selected from the group consisting of a *T. gondii* genomic expression library and a *T. gondii* cDNA expression library with an antiserum, wherein said antiserum is selected from the group consisting of antiserum raised against *T. gondii* oocysts, antiserum raised against *T. gondii* bradyzoites,
- antiserum raised against *T. gondii* infected cat gut, and antiserum isolated from a cat immune to *T. gondii* infection; and b) identifying a protein expressed from said library that selectively binds to antibodies in said antiserum.
 - 4. An isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEO ID

NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:50, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID 10 NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID 15 NO:273, SEQ ID NO:338, SEQ ID NO:340, and SEQ ID NO:343.

- 5. An isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene comprising a nucleic acid molecule selected from the group consisting of nTG2₃₃₉, nTG4₅₂₆, nTG4₁₄₇₈, nTG5₆₅₇, nTG5₁₀₂₉, nTG6₄₂₅, nTG7₄₁₇, nTG8₅₀₇, nTG9₇₁₈, nTG10₄₄₁, nTG11₄₂₈, nTG13₂₈₂, nTG15₃₀₄, nTG16₂₈₄, nTG17₆₉₀, nTG18₃₁₃,
- $\begin{array}{lll} 20 & nTG9_{718}, \, nTG10_{441}, \, nTG11_{428}, \, nTG13_{282}, \, nTG15_{304}, \, nTG16_{284}, \, nTG17_{690}, \, nTG18_{313}, \\ & nTG19_{389}, \, nTG21_{548}, \, nTG22_{310}, \, nTG23_{220}, \, nTG24_{642}, \, nTG25_{381}, \, nTG26_{432}, \, nTG27_{282}, \\ & nTG28_{466}, \, nTG30_{539}, \, nTG31_{1233}, \, nTG32_{411}, \, nTG33_{441}, \, nTG34_{491}, \, nTG35_{387}, \, nTG36_{417}, \\ & nTG37_{416}, \, nTG38_{500}, \, nTG40_{321}, \, nTG41_{513}, \, nTG42_{528}, \, nTG43_{375}, \, nTG44_{543}, \, nTG45_{573}, \\ & nTG46_{1835}, \, nTG48_{604}, \, nTG48_{549}, \, nTG49_{270}, \, nTG50_{306}, \, nTG51_{804}, \, nTG52_{867}, \, nTG53_{1434}, \end{array}$
- $\begin{array}{lll} 25 & nTG54_{680}, nTG55_{296}, nTG56_{723}, nTG57_{270}, nTG58_{503}, nTG60_{322}, nTG61_{390}, nTG62_{699}, \\ & nTG63_{419}, nTG64_{303}, nTG65_{696}, nTG66_{173}, nTG67_{369}, nTG68_{566}, nTG69_{616}, nTG70_{762}, \\ & nTG71_{236}, nTG72_{569}, nTG73_{232}, nTG74_{276}, nTG75_{309}, nTG76_{534}, nTG76_{423}, nTG77_{327}, \\ & nTG78_{444} \ and \ nTG79_{928}. \end{array}$
- 6. An isolated immunogenic protein encoded by a nucleic acid molecule that
 30 hybridizes under stringent hybridization conditions with a gene comprising the

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complement of a nucleic acid sequence selected from the group consisting of SEO ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEO ID NO:11. SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:28, SEO ID NO:30, SEO ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:50, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:87, SEQ ID 10 NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID 15 NO:138, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:340, and **SEQ ID NO:343.**

7. An isolated immunogenic protein encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene comprising a nucleic 20 acid molecule selected from the group consisting of nTG2₃₃₉, nTG4₅₂₆, nTG4₁₄₇₈, $nTG5_{657}$, $nTG5_{1029}$, $nTG6_{425}$, $nTG7_{417}$, $nTG8_{507}$, $nTG9_{718}$, $nTG10_{441}$, $nTG11_{428}$, $nTG13_{282}$ nTG15₃₀₄, nTG16₂₈₄, nTG17₆₉₀, nTG18₃₁₃, nTG19₃₈₉, nTG21₅₄₈, nTG22₃₁₀, nTG23₂₂₀, $nTG24_{642}$, $nTG25_{381}$, $nTG26_{432}$, $nTG27_{282}$, $nTG28_{466}$, $nTG30_{539}$, $nTG31_{1233}$, $nTG32_{411}$, nTG33₄₄₁, nTG34₄₉₁, nTG35₃₈₇, nTG36₄₁₇, nTG37₄₁₆, nTG38₅₀₀, nTG40₃₂₁, nTG41₅₁₃, 25 $nTG42_{528}$, $nTG43_{375}$, $nTG44_{543}$, $nTG45_{573}$, $nTG46_{1835}$, $nTG48_{604}$, $nTG48_{549}$, $nTG49_{270}$, $nTG50_{306}$, $nTG51_{804}$, $nTG52_{867}$, $nTG53_{1434}$, $nTG54_{680}$, $nTG55_{296}$, $nTG56_{723}$, $nTG57_{270}$, $nTG58_{503}$, $nTG60_{322}$, $nTG61_{390}$, $nTG62_{699}$, $nTG63_{419}$, $nTG64_{303}$, $nTG65_{696}$, $nTG66_{173}$, nTG67₃₆₉, nTG68₅₆₆, nTG69₆₁₆, nTG70₇₆₂, nTG71₂₃₆, nTG72₅₆₉, nTG73₂₃₂, nTG74₂₇₆, nTG75₃₀₉, nTG76₅₃₄, nTG76₄₂₃, nTG77₃₂₇, nTG78₄₄₄ and nTG79₉₂₈. 30

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8. A composition to inhibit T. gondii oocyst shedding in a cat due to infection with T. gondii, said composition comprising a compound selected from the group consisting of: an isolated immunogenic T. gondii protein encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene comprising the complement of a nucleic acid sequence selected from the group consisting of SEQ ID 5 NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEO ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEO ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:52, SEQ ID 10 NO:54, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:50, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68, SEO ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID 15 NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEO ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID 20 NO:138, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:340, and SEQ ID NO:343; an isolated antibody that selectively binds to said immunogenic T. gondii protein; and an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene comprising a nucleic acid sequence selected from 25 the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEO ID 30 NO:48, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:57, SEO ID

NO:50. SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67. SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76. SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:85. SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95. SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105. SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115. SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125. SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:134. SEQ ID NO:136, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143. SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273. SEQ ID NO:340, and SEQ ID NO:343.

A method to inhibit T. gondii oocyst shedding in a cat due to infection with T. 9. gondii, said method comprising administering to said cat a composition comprising a compound selected from the group consisting of: a) an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene comprising a nucleic 15 acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO.5. SI Q ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15. N () ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25. NO 1D NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34. NO:10 NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID 20 NO:44, NO:45, NO:46, SEQ ID NO:48, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:50, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72. SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, St Q ID NO:84, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID 25 NO:91. SEQ ID NO:95, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101. N. Q. ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, St Q ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SLQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID NO:138, SEQ ID 30

NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:265, SEQ ID NO:267, SEO ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:282, SEO ID NO:292, SEO ID NO:306, SEQ ID NO:311, SEQ ID NO:338, SEQ ID NO:340, and SEQ ID NO:343. b) an isolated immunogenic T. gondii protein encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene comprising the complement of a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11. SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEO ID NO:21, SEO ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID 10 NO:32, SEO ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:52, SEO ID NO:54, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:50, SEQ ID NO:59, SEO ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID 15 NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:87, SEO ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEO ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEO ID 20 NO:129, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEO ID NO:138, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:282, SEQ ID NO:292, SEQ ID NO:306, SEQ ID NO:311, SEQ ID NO:338, SEQ ID NO:340, and SEQ ID NO:343; and c) an isolated antibody that selectively binds to said immunogenic 25 T. gondii protein.

- 10. The invention of Claim 1, 2 or 3 wherein said antiserum isolated from a cat immune to *T. gondii* infection is enriched for antibodies to *T. gondii* gametogenic stages.
- 11. An isolated antibody that selectively binds to a protein as set forth in Claim 3, 6 or 7.

- The nucleic acid molecule of Claim 1, 4 or 5, wherein said nucleic acid molecule 12. hybridizes under stringent hybridization conditions with a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEO ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:50, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68, SEQ ID 10 NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID 15 NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:340, and 20 **SEQ ID NO:343.**
 - 13. The nucleic acid molecule of Claim 4 or 5, wherein said nucleic acid molecule encodes an immunogenic *T. gondii* protein.
- 14. The nucleic acid molecule of Claim 1, 4 or 5, wherein said nucleic acid molecule comprises a nucleic acid sequence that is at least about 75% identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:55, SEQ ID NO:55, SEQ ID NO:55, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:55, SEQ ID NO:

- NO:57. SEQ ID NO:50, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65. SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74. SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84. SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93. SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103. SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113. SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123. SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:132. SEQ ID NO:134, SEQ ID NO:136, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:141. SEQ ID NO:143, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271. SEQ ID NO:273, SEQ ID NO:340, and SEQ ID NO:343.
 - 15. The nucleic acid molecule of Claim 1, 4 or 5, wherein said nucleic acid molecule is selected from the group consisting of: a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ
- 15 ID NO 5. SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15. SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25. SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34. SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44. SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:54, SEQ ID NO:54, SEQ ID NO:54, SEQ ID NO:554, SEQ ID N
- 20 NO:55. SEQ ID NO:57, SEQ ID NO:50, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63. SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72. SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82. SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91. SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:91. SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID
- 25 NO:101. SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111. SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121. SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131. SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID NO:138, SEQ ID NO:139. SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:139. SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:150.
- 30 NO:269. SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:282, SEQ ID NO:292, SEQ ID

NO:306, SEQ ID NO:311, SEQ ID NO:338, SEQ ID NO:340, and SEQ ID NO:343.; and a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEO ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:50, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID 10 NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEO ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID 15 NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEO ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:282, SEQ ID NO:292, SEQ ID NO:306, SEQ ID NO:311, SEQ ID NO:338, SEQ ID NO:340, and SEQ ID NO:343...

- 16. The nucleic acid molecule of Claim1, 4 or 5, wherein said nucleic acid molecule encodes a protein comprising an amino acid sequence that is at least about 75% identical to an amino acid sequence encoded by a nucleic acid molecule selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID
- NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:50, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID

NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:282, SEQ ID NO:292, SEQ ID NO:306, SEQ ID NO:311, SEQ ID NO:338, SEQ ID NO:340, and SEO ID NO:343.

- 17. The nucleic acid molecule of Claim 1, 4 or 5, wherein said nucleic acid molecule hybridizes under stringent hybridization conditions with a nucleic acid molecule comprising a nucleic acid sequence encoding a protein comprising an amino acid sequence selected from the group consisting of SEO ID NO.2. SEO ID NO.4. SEO ID NO.4.
- sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID
- 20 NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID
- NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137, SEQ ID NO:140, SEQ ID NO:142, SEQ ID NO:144, SEQ ID NO:266, SEQ ID NO:268, SEQ ID NO:270, SEQ ID NO:272, SEQ ID
- 30 NO:274, SEQ ID NO:283, SEQ ID NO:293, SEQ ID NO:307, SEQ ID NO:312, and SEQ ID NO:341.

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- 18. The nucleic acid molecule of Claim 1, 4 or 5, wherein said nucleic acid molecule is selected from the group consisting of: a nucleic acid molecule comprising a nucleic acid sequence that encodes a protein having an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ
- 5 ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID
- 10 NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID
- NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137, SEQ ID NO:140, SEQ ID NO:142, SEQ ID NO:144, SEQ ID NO:266, SEQ ID NO:268, SEQ ID NO:270, SEQ ID NO:272, SEQ ID NO:274, SEQ ID NO:283, SEQ ID NO:293, SEQ ID NO:307, SEQ ID NO:312, and SEQ ID NO:341;
- and a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule encoding a protein having any of said amino acid sequences.
 - 19. The nucleic acid molecule of Claim1, 4 or 5, wherein said nucleic acid molecule comprises an oligonucleotide.
 - 20. A recombinant molecule comprising a nucleic acid molecule as set forth in Claim1, 4 or 5, operatively linked to a transcription control sequence.
 - 21. A recombinant virus comprising a nucleic acid molecule as set forth in Claim 1, 4 or 5.
 - 22. A recombinant cell comprising a nucleic acid molecule as set forth in Claim 1, 4 or 5.

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- 23. The nucleic acid molecule of Claim 5, wherein said nucleic acid molecule hybridizes under stringent hybridization conditions with a nucleic acid molecule selected from the group consisting of nTG2₃₃₉, nTG4₅₂₆, nTG4₁₄₇₈, nTG5₆₅₇, nTG5₁₀₂₉, nTG6₄₂₅, nTG7₄₁₇, nTG8₅₀₇, nTG9₇₁₈, nTG10₄₄₁, nTG11₄₂₈, nTG13₂₈₂, nTG15₃₀₄, nTG16₂₈₄, nTG17₆₉₀, nTG18₃₁₃, nTG19₃₈₉, nTG21₅₄₈, nTG22₃₁₀, nTG23₂₂₀, nTG24₆₄₂, nTG25₃₈₁, nTG26₄₃₂, nTG27₂₈₂, nTG28₄₆₆, nTG30₅₃₉, nTG31₁₂₃₃, nTG32₄₁₁, nTG33₄₄₁, nTG34₄₉₁, nTG35₃₈₇, nTG36₄₁₇, nTG37₄₁₆, nTG38₅₀₀, nTG40₃₂₁, nTG41₅₁₃, nTG42₅₂₈, nTG43₃₇₅, nTG44₅₄₃, nTG45₅₇₃, nTG46₁₈₃₅, nTG48₆₀₄, nTG48₅₄₉, nTG49₂₇₀, nTG50₃₀₆, nTG51₈₀₄, nTG52₈₆₇, nTG53₁₄₃₄, nTG54₆₈₀, nTG55₂₉₆, nTG56₇₂₃, nTG57₂₇₀, nTG58₅₀₃, nTG60₃₂₂, nTG61₃₉₀, nTG62₆₉₉, nTG63₄₁₉, nTG64₃₀₃, nTG65₆₉₆, nTG66₁₇₃, nTG67₃₆₉, nTG68₅₆₆, nTG69₆₁₆, nTG70₇₆₂, nTG71₂₃₆, nTG72₅₆₉, nTG73₂₃₂, nTG74₂₇₆, nTG75₃₀₉, nTG76₅₃₄, nTG76₄₂₃, nTG77₃₂₇, nTG78₄₄₄, nTG79₉₂₈, nTG73₂₃₂, nTG64₃₀₃, nTG71_{236a}, nTG66_{425a}, nTG76₄₂₃, nTG77₃₂₇, nTG78₄₄₄, nTG79₉₂₈, nTG22_{310a}, nTG64₃₀₃, nTG71_{236a}, nTG6_{425a}, nTG71₃₁₉₇, nTG7₁₃₉₇, nTG7₁₂₈₅.
- 24. The nucleic acid molecule of Claim 1, 4 or 5, wherein said nucleic acid molecule is at least about 75% identical to a nucleic acid molecule selected from the group consisting of nTG2₃₃₉, nTG4₅₂₆, nTG4₁₄₇₈, nTG5₆₅₇, nTG5₁₀₂₉, nTG6₄₂₅, nTG7₄₁₇, nTG8₅₀₇, nTG9₇₁₈, nTG10₄₄₁, nTG11₄₂₈, nTG13₂₈₂, nTG15₃₀₄, nTG16₂₈₄, nTG17₆₉₀, nTG18₃₁₃, nTG19₃₈₉, nTG21₅₄₈, nTG22₃₁₀, nTG23₂₂₀, nTG24₆₄₂, nTG25₃₈₁, nTG26₄₃₂, nTG27₂₈₂, nTG28₄₆₆, nTG30₅₃₉, nTG31₁₂₃₃, nTG32₄₁₁, nTG33₄₄₁, nTG34₄₉₁, nTG35₃₈₇, nTG36₄₁₇,
 20 nTG37₄₁₆, nTG38₅₀₀, nTG40₃₂₁, nTG41₅₁₃, nTG42₅₂₈, nTG43₃₇₅, nTG44₅₄₃, nTG45₅₇₃, nTG46₁₈₃₅, nTG48₆₀₄, nTG48₅₄₉, nTG49₂₇₀, nTG50₃₀₆, nTG51₈₀₄, nTG52₈₆₇, nTG53₁₄₃₄, nTG54₆₈₀, nTG55₂₉₆, nTG56₇₂₃, nTG57₂₇₀, nTG58₅₀₃, nTG60₃₂₂, nTG61₃₉₀, nTG62₆₉₉, nTG63₄₁₉, nTG64₃₀₃, nTG65₅₀₆, nTG66₃₁₃, nTG66₄₃₃, nTG66₄₃₃, nTG66₄₂₃, nTG76₄₂₃, nTG77₃₂₇,
 25 nTG78₄₄₄, nTG79₉₂₈, nTG22_{310a}, nTG64_{303a}, nTG71₂₃₆, nTG64_{25a}, nTG41_{513a}, nTG₈₆₇.
 - 25. The nucleic acid molecule of Claim 1, 4 or 5, wherein said nucleic acid molecule is selected from the group consisting of: a nucleic acid molecule selected from the group consisting of nTG2₃₃₉, nTG4₅₂₆, nTG4₁₄₇₈, nTG5₆₅₇, nTG5₁₀₂₉, nTG6₄₂₅, nTG7₄₁₇, nTG8₅₀₇,
- 30 nTG9₇₁₈, nTG10₄₄₁, nTG11₄₂₈, nTG13₂₈₂, nTG15₃₀₄, nTG16₂₈₄, nTG17₆₉₀, nTG18₃₁₃,

nTG₁₃₉₇, nTG₁₇₈₅.

 $nTG19_{389}, nTG21_{548}, nTG22_{310}, nTG23_{220}, nTG24_{642}, nTG25_{381}, nTG26_{432}, nTG27_{282}, nTG27_{282}, nTG28_{381}, nTG28_{381}, nTG28_{382}, nTG$ $nTG28_{466}$, $nTG30_{539}$, $nTG31_{1233}$, $nTG32_{411}$, $nTG33_{441}$, $nTG34_{491}$, $nTG35_{387}$, $nTG36_{417}$ nTG37₄₁₆, nTG38₅₀₀, nTG40₃₂₁, nTG41₅₁₃, nTG42₅₂₈, nTG43₃₇₅, nTG44₅₄₃, nTG45₅₇₃, $nTG46_{1835}, nTG48_{604}, nTG48_{549}, nTG49_{270}, nTG50_{306}, nTG51_{804}, nTG52_{867}, nTG53_{1434},$ $nTG54_{680}$, $nTG55_{296}$, $nTG56_{723}$, $nTG57_{270}$, $nTG58_{503}$, $nTG60_{322}$, $nTG61_{390}$, $nTG62_{699}$ $nTG63_{419},\,nTG64_{303},\,nTG65_{696},\,nTG66_{173},\,nTG67_{369},\,nTG68_{566},\,nTG69_{616},\,nTG70_{762},\,nTG69_{616},\,nTG70_{762},\,nTG69_{616},\,nTG70_{762},\,nTG69_{616},\,nTG70_{762},\,nTG69_{616},\,nTG70_{762},\,nTG69_{616},\,nTG70_{762},\,nTG69_{616},\,nTG70_{762},\,nTG69_{616},\,nTG70_{762},\,nTG69_{616},\,nTG70_{762},\,nTG69_{616},\,nTG69_{616},\,nTG70_{762},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG69_{616},\,nTG$ $nTG71_{236},\,nTG72_{569},\,nTG73_{232},\,nTG74_{276},\,nTG75_{309},\,nTG76_{534},\,nTG76_{423},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG77_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_$ $nTG78_{444},\,nTG79_{928},nTG22_{310a},\,nTG64_{303a},\,nTG71_{236a},\,nTG6_{425a},\,nTG41_{513a},\,nTG_{867},\,nTG71_{236a},\,nTG6_{425a},\,nTG41_{513a},\,nTG_{867},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a},\,nTG71_{236a$ nTG₁₃₉₇, nTG₁₇₈₅; and a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule selected from the group consisting of nTG2339, nTG4526, nTG41478, 10 $nTG5_{657}$, $nTG5_{1029}$, $nTG6_{425}$, $nTG7_{417}$, $nTG8_{507}$, $nTG9_{718}$, $nTG10_{441}$, $nTG11_{428}$, $nTG13_{282}$ nTG15₃₀₄, nTG16₂₈₄, nTG17₆₉₀, nTG18₃₁₃, nTG19₃₈₉, nTG21₅₄₈, nTG22₃₁₀, nTG23₂₂₀, $nTG24_{642}, nTG25_{381}, nTG26_{432}, nTG27_{282}, nTG28_{466}, nTG30_{539}, nTG31_{1233}, nTG32_{411},$ $nTG33_{441}, nTG34_{491}, nTG35_{387}, nTG36_{417}, nTG37_{416}, nTG38_{500}, nTG40_{321}, nTG41_{513},$ 15 $nTG42_{528}$, $nTG43_{375}$, $nTG44_{543}$, $nTG45_{573}$, $nTG46_{1835}$, $nTG48_{604}$, $nTG48_{549}$, $nTG49_{270}$, nTG50₃₀₆, nTG51₈₀₄, nTG52₈₆₇, nTG53₁₄₃₄, nTG54₆₈₀, nTG55₂₉₆, nTG56₇₂₃, nTG57₂₇₀, nTG58₅₀₃, nTG60₃₂₂, nTG61₃₉₀, nTG62₆₉₉, nTG63₄₁₉, nTG64₃₀₃, nTG65₆₉₆, nTG66₁₇₃, nTG67₃₆₉, nTG68₅₆₆, nTG69₆₁₆, nTG70₇₆₂, nTG71₂₃₆, nTG72₅₆₉, nTG73₂₃₂, nTG74₂₇₆, nTG75₃₀₉, nTG76₅₃₄, nTG76₄₂₃, nTG77₃₂₇, nTG78₄₄₄, nTG79₉₂₈, nTG22_{310a}, nTG64_{303a}, 20 nTG71_{236a}, nTG6_{425a}, nTG41_{513a}, nTG₈₆₇, nTG₁₃₉₇, nTG₁₇₈₅. The nucleic acid molecule of Claim 1, 4 or 5, wherein said nucleic acid molecule 26. encodes a protein comprising an amino acid sequence that is at least about 75% identical to an amino acid sequence encoded by a nucleic acid molecule selected from the group consisting of nTG2₃₃₉, nTG4₅₂₆, nTG4₁₄₇₈, nTG5₆₅₇, nTG5₁₀₂₉, nTG6₄₂₅, nTG7₄₁₇, nTG8₅₀₇, $nTG9_{718}, nTG10_{441}, nTG11_{428}, nTG13_{282}, nTG15_{304}, nTG16_{284}, nTG17_{690}, nTG18_{313}, nTG1$ 25 $nTG19_{389}$, $nTG21_{548}$, $nTG22_{310}$, $nTG23_{220}$, $nTG24_{642}$, $nTG25_{381}$, $nTG26_{432}$, $nTG27_{282}$ $nTG28_{466}$, $nTG30_{539}$, $nTG31_{1233}$, $nTG32_{411}$, $nTG33_{441}$, $nTG34_{491}$, $nTG35_{387}$, $nTG36_{417}$ nTG37₄₁₆, nTG38₅₀₀, nTG40₃₂₁, nTG41₅₁₃, nTG42₅₂₈, nTG43₃₇₅, nTG44₅₄₃, nTG45₅₇₃, $nTG46_{1835}, nTG48_{604}, nTG48_{549}, nTG49_{270}, nTG50_{306}, nTG51_{804}, nTG52_{867}, nTG53_{1434}, nTG50_{1835}, nTG50_$ 30 $nTG54_{680}$, $nTG55_{296}$, $nTG56_{723}$, $nTG57_{270}$, $nTG58_{503}$, $nTG60_{322}$, $nTG61_{390}$, $nTG62_{699}$

 $nTG63_{419}, nTG64_{303}, nTG65_{696}, nTG66_{173}, nTG67_{369}, nTG68_{566}, nTG69_{616}, nTG70_{762}, \\ nTG71_{236}, nTG72_{569}, nTG73_{232}, nTG74_{276}, nTG75_{309}, nTG76_{534}, nTG76_{423}, nTG77_{327}, \\ nTG78_{444}, nTG79_{928}, nTG22_{310a}, nTG64_{303a}, nTG71_{236a}, nTG6_{425a}, nTG41_{513a}, nTG_{867}, \\ nTG_{1397}, nTG_{1785}.$

- 5 27. The immunogenic protein of Claim 3, 6 or 7, wherein said protein is selected from the group consisting of: a protein comprising an amino acid sequence that is at least about 75% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEO ID NO:14, SEO ID NO:16, SEO ID NO:18, SEO ID NO:20, SEO ID NO:22, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID 10 NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEO ID NO:77, SEO ID NO:79, SEO ID NO:81, SEQ ID NO:83, SEO ID 15 NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:133, SEQ ID NO:135, SEQ ID 20 NO:137, SEQ ID NO:140, SEQ ID NO:142, SEQ ID NO:144, SEQ ID NO:266, SEQ ID NO:268, SEQ ID NO:270, SEQ ID NO:272, SEQ ID NO:274, SEQ ID NO:283, SEQ ID NO:293, SEQ ID NO:307, SEQ ID NO:312, and SEQ ID NO:341; and a protein comprising an epitope of said protein having said amino acid sequence.
- 25 28. The immunogenic protein of Claim 3, 6 or 7, wherein said protein is selected from the group consisting of: an immunogenic protein encoded by a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID

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29. The immunogenic protein of Claim 3, 6 or 7, wherein said protein is selected from the group consisting of: an immunogenic protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID 20 NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEO ID NO:16. SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:56, SEQ ID NO:58, SEQ ID 25 NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEO ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID

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- 5 SEQ ID NO:341.; and an immunogenic *T. gondii* protein encoded by an allelic variant of a nucleic acid molecule encoding a protein comprising any of said amino acid sequences.
 - 30. The immunogenic protein of Claim 3, 6 or 7, wherein said protein comprises an epitope that elicits an immune response.
- 31. The immunogenic protein of Claim 3, 6 or 7, wherein said protein is selected 10 from the group consisting of: an immunogenic protein encoded by a nucleic acid molecule selected from the group consisting of:nTG2₃₃₉, nTG4₅₂₆, nTG4₁₄₇₈, nTG5₆₅₇, $nTG5_{1029}$, $nTG6_{425}$, $nTG7_{417}$, $nTG8_{507}$, $nTG9_{718}$, $nTG10_{441}$, $nTG11_{428}$, $nTG13_{282}$, $nTG15_{304}$, nTG16₂₈₄, nTG17₆₉₀, nTG18₃₁₃, nTG19₃₈₉, nTG21₅₄₈, nTG22₃₁₀, nTG23₂₂₀, nTG24₆₄₂, nTG25₃₈₁, nTG26₄₃₂, nTG27₂₈₂, nTG28₄₆₆, nTG30₅₃₉, nTG31₁₂₃₃, nTG32₄₁₁, nTG33₄₄₁, $nTG34_{491}, nTG35_{387}, nTG36_{417}, nTG37_{416}, nTG38_{500}, nTG40_{321}, nTG41_{513}, nTG42_{528},$ 15 $nTG43_{175}$, $nTG44_{543}$, $nTG45_{573}$, $nTG46_{1835}$, $nTG48_{604}$, $nTG48_{549}$, $nTG49_{270}$, $nTG50_{306}$ nTG51₈₀₄, nTG52₈₆₇, nTG53₁₄₃₄, nTG54₆₈₀, nTG55₂₉₆, nTG56₇₂₃, nTG57₂₇₀, nTG58₅₀₃, $nTG60_{322}$, $nTG61_{390}$, $nTG62_{699}$, $nTG63_{419}$, $nTG64_{303}$, $nTG65_{696}$, $nTG66_{173}$, $nTG67_{369}$ nTG68₅₆₆, nTG69₆₁₆, nTG70₇₆₂, nTG71₂₃₆, nTG72₅₆₉, nTG73₂₃₂, nTG74₂₇₆, nTG75₃₀₉, nTG76₅₃₄, nTG76₄₂₃, nTG77₃₂₇, nTG78₄₄₄, nTG79₉₂₈ nTG22_{310a}, nTG64_{303a}, nTG71_{236a}, 20 nTG6_{425a}, nTG41_{513a}, nTG₈₆₇, nTG₁₃₉₇, nTG₁₇₈₅; and an immunogenic protein encoded by a nucleic acid molecule comprising an allelic variant of any of said nucleic acid molecules.
 - 32. The invention of Claim 8 or 9, wherein said composition further comprises a component selected from the group consisting of an excipient, an adjuvant, and a carrier.
 - 33. The invention of Claim 8 or 9, wherein said compound is selected from the group consisting of a genetic vaccine, a recombinant virus vaccine and a recombinant cell vaccine.
- 34. The invention of Claim 8 or 9, wherein said compound comprises a recombinant 30 molecule.

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- 35. The invention of Claim 8 or 9, wherein said compound is selected from the group consisting of a recombinant virus genome and a recombinant plasmid.
- 36. The invention of Claim 8 or 9, wherein said composition is administered by a method selected from the group consisting of injection, oral administration, inhalation, nasal administration, intraocular administration, anal administration, topical administration, particle bombardment, and intradermal scarification.
- 37. The invention of Claim 8 or 9, wherein said composition is administered by a method selected from the group consisting of intradermal injection and intramuscular injection.
- 10 38. The invention of Claim 8 or 9, wherein said composition is administered mucosally
 - 39. A method to detect parasite cysts or oocysts in feces, said method comprising:
 - 1 contacting a sample of feces with a solid support capable of binding oocysts;
 - 2 allowing the sample to dry onto the solid support;
- 3 washing the sample on the solid support with an aqueous wash solution:
 - adding an aqueous elution solution to the sample and eluting DNA from the sample into the aqueous elution solution by heating;
 - 5 ICR amplifying oocyst-specific DNA with primers specific to the oocyst being detected, and
- 20 6 detecting the presence of a PCR amplification product resulting from amplification of excepts-specific DNA in the sample wherein the presence of said product indicates the presence of cysts or oocysts in said feces.
 - 40. A metiest according to Claim 39, wherein the sample of feces is solubilized in an aqueous solution before contacting the sample with a solid support capable of binding oocysts
 - 41. A method according to Claim 39, wherein the aqueous wash solution comprises distilled water
 - 42. A method according to Claim 39, wherein the aqueous elution solution comprises distilled water

- 43. A method according to Claim 39, wherein the heating step comprises heating to approximately 95° C.
- 44. A method according to Claim 39, wherein the solid support capable of binding oocysts comprises paper.
- 5 45. A method according to Claim 39, wherein the solid support comprises one or more compounds capable of binding inhibitors of PCR amplification.
 - 46. The method of Claim 39, wherein the parasite oocysts are enteric apicomplexa oocysts.
- The method of Claim 46 wherein the enteric apicomplexa oocysts are selected from the group consisting of *Cryptosporidium* oocysts and *Toxoplasma* oocysts.

SEQUENCE LISTING

<110> Milhausen, Michael James Lutz, Susan Bektesh Ng, Ray K.

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<140> 08/994,825

<141> 1997-12-19

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Trp Ile Pro Ser Gly Ser Val Leu Leu Ser Ser Ser Pro Ala His Ser
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gcc ggt cct cgg aat act cga acg tct cga gtt gcg cgc gtt ggc ctg 144
Ala Gly Pro Arg Asn Thr Arg Thr Ser Arg Val Ala Arg Val Gly Leu
35 40 45

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Asp 65	Glu	Gly	.ter	Asp	Val 70	Arg	Gly	Arg	Phe	Phe 75	Arg	Gly	Arg	Gln	Thr 80	
		: ::											-			288
		111														336
		· ;				_										357
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Ala	G!.	į.	:	Aist.	Thr	Arg	Thr 40	Ser	Arg	Val	Ala	Arg 45	Val	Gly	Leu	
Glu	Al i	•	•	a	Lys	Gly 55	Glu	Thr	Glu	Ser	Arg 60	Arg	Ser	Arg	Gln	
Asp 65	G:			:	Val 70	Arg	Gly	Arg	Phe	Phe 75	Arg	Gly	Arg	Gln	Thr 80	
Gly	A. 1			. • •	His	Met	Gly	Val	Туг 90	Glu	Gly	His	Asp	Gly 95	Asn	
Glu	Etie				Arg	Glu	Gln	Gly 105	Ala	Cys	Asp	Phe	Ser 110	Ala	Ser	
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20 25 . 30

Lys Pro Ser Leu Phe Ala Ala Ser Glu Leu Pro Pro Asp Glu Thr Ala 35 40 45

Asp Ser Gly Asp Thr Gly Pro Phe Arg Arg Asp Arg Asp Phe Phe Ala 50 55 60

Gly Thr Ala Gly Glu His Asp Ala Ser Ala Met Arg Asp Lys Glu Ala 65 7C 75 80

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tcc ccc atc gac gaa aac cct gca gag atg gaa agc acc atc tcc gag 144
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35 40 45

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Gly Glu Ala Gly Ser Ala Val Ala Ala Pro Glu Gln Gly Ile Gln Pro 50 55 60

Glu Ala Glu Phe Ala Thr Ala Ser Glu Glu Pro Arg Pro Leu Glu Pro 65 70 75 80

Val Asp Pro Glu Met Ala Ala Gln Gln Pro Gln Leu Pro Gln Glu Ala 85 . 90 95

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					gag Glu							-				387
					tcg Ser									_		435
					cat His 145											483
					aac Asn					-		_	_	_		531
					ctt Leu											579
					ccc Pro							-	_	-	-	627
					gaa Glu									-		675
					gca Ala 225											723
					gac Asp											771
					cca Pro											819
ccc	aga	atg	cgc	aat	gct	ctc	gaa	ccc	tct	gcc	aag	gtc	ctc	gaa	ccg	867

Pro	Arg	Met 270	Arg	Asn	Ala	Leu	Glu 275	Pro	Ser	Ala	Lys	Val 280	Leu	Glu	Pro	
														act Thr	-	915
-			_	-					-	_	-	_	_	gat Asp	_	963
	_	-	_	-				-	-			-		acc Thr 330		1011
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- Asp Thr Asp Asn Ser Glu Ala Asp Ser Gln Gln Glu Asp Asp Ser Val 65 70 75 80
- Gly Glu Asp Ser Phe Leu Gln Glu Glu Glu Glu Glu Glu Glu 85 90 95
- Arg Ala Val Glu Asp Pro Tyr Ala Ala Ala Glu Pro Ser Tyr Leu Glu 100 105 110
- Glu Asp Asn Thr Val Asp Asp Ser Ala Ala Glu Asp Tyr Ala Pro Ala 115 120 125
- Ser Phe Val Gln Ile Gly Ser Gly Glu Arg Lys Ile Arg Ala His Met 130 135 140
- His Leu Asp Ser Arg Gln Val Ala Pro Glu Arg Phe Ala His Ala Phe 145 150 155 160
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- Leu Leu Asp Glu Ala Ala Pro Gly Gly Gly Ala Ser Ala Val Ser 180 185 190
- Pro Ile Asp Glu Asn Pro Ala Glu Met Glu Ser Thr Ile Ser Glu Gly
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- Glu Ala Gly Ser Ala Val Ala Ala Pro Glu Gln Gly Ile Gln Pro Glu 210 215 220
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Ser Pro Ala Leu Val Pro Pro Ala Glu Thr Glu Glu Gly Thr Ala Ala 290 295 300

Gln Ile Ala Glu Glu Met Ser Lys Gln Asp Gln Gly Met Gln Glu Ala 305 310 315 320

Arg Pro Gln Glu Val Leu Thr Arg His Thr Trp Gln Asp Met Glu Arg 325 330 335

Thr Glu Asp Leu Arg Lys Asn Asp Val Pro Ala Ala Val Ala Asn Ser 340 345 350

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Pro Phe Ala Glu Leu Pro Gln Leu Ala Glu Ile Pro Lys Leu Ser Glu
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gcc gag atg ccc aag tta tca gat ata ccc aag atg gct gag atg ccc 192

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		toa Sm:														240
		ao 1 Lys														288
		ati; Mot														336
		C_G i: 11'														384
		adri Ari														432
		o≛ : M⊷≐														480
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tca Ser		:					gac Asp 200									624
		‡ 7 :					ttc Phe									657
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Lys Leu Ser Asp Ile Pro Lys Met Ala Glu Met Pro Lys Leu Ser Asp 65 70 75 80

Ile Pro Lys Met Ala Glu Met Pro Lys Phe Ser Asp Ile Pro Lys Met 85 90 95

Ala Glu Met Pro Lys Leu Ser Asp Met Pro Arg Met Ala Asp Ile Pro 100 105 110

Gln Phe Pro Glu Met Pro Arg Met Val Asp Met Pro Gln Phe Pro Glu 115 120 125

Ile Pro Arg Met Ala Asp Met Arg Arg Phe Pro Glu Met Ser Lys Ile 130 135 140

Ala Asp Met Pro Lys Phe Pro Asp Met Pro Asn Val Thr Glu Met Pro 145 150 155 160

Lys Leu Ala Asp Leu Pro Arg Leu Ala Asp Met Pro Ser Ile Ala Asp 165 170 175

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Leu Pro Lys Ile Ala Asp Met Pro Lys Phe Ser Asp Met Pro Lys Met
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gcc gag atg ccc aag tta tca gat ata ccc aag atg gct gag atg ccc 192
Ala Glu Met Pro Lys Leu Ser Asp Ile Pro Lys Met Ala Glu Met Pro
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ata ccc aag atg gct gag atg ccc aag ttt tca gat ata ccc aag atg 288

Ile Pro Lys Met Ala Glu Met Pro Lys Phe Ser Asp Ile Pro Lys Met

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Ile Pro Arg Met Ala Asp Met Arg Arg Phe Pro Glu Met Ser Lys Ile

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Ala Asp Met Pro Lys Phe Pro Asp Met Pro Asn Val Thr Glu Met Pro
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aag ctt gca gat ttg cca agg ctt gct gac atg ccc agt att gcc gac 528

WO 99/32633																
Lys	Leu	Ala	Asp	Leu 165	Pro	Arg	Leu	Ala	Asp 170	Met	Pro	Ser	Ile	Ala 175	Asp	
,		ogi Ari			_	_		٠.		-	-	_				576
	_	ata Lie 14		_		-										624
-		a : A. i														672
_		n.		•												720
		 i:		-												768
_																816
gct Ala		. 1 2 1	1,1	itgti	atati	tg a	caaa	tggc1	t gta	atcto	ccat	agti	tata	gtg		869
agg	aats	• .•	. 1 3	tat	tc c	gagg	actc	t ata	actg	aacc	cgc	ggca	tac (gagga	aaactg	929
aca	aqtt	::*	• • •	· 1:g	tt t	ctga	tctt	c cc	cgaa	aaga	aaa	aaaa	atg	accg	tcttaa	989
aaa	ddu +	,		. 1-1	a a	aaaa	aaaa	a aa	aaaa	aaaa						1029
<21 <21	.0> ; .1> ; .2> ; .3> ~	· _ •		; ;	ndii											
	00> : a Mc* L			::0	_	Arg	Gln	Thr	Gly 10	_	Leu	. Ser	Asp	Leu 15	Pro	
Pro	o Phe	· i	•	. 10	Pro	Glr	Lei	Ala 25		ılle	Pro	Lys	Leu 30		Glu	

Leu Pro Lys Ile Ala Asp Met Pro Lys Phe Ser Asp Met Pro Lys Met 35 40 45

- Ala Glu Met Pro Lys Leu Ser Asp Ile Pro Lys Met Ala Glu Met Pro 50 55 60
- Lys Leu Ser Asp Ile Pro Lys Met Ala Glu Met Pro Lys Leu Ser Asp 65 70 75 80
- Ile Pro Lys Met Ala Glu Met Pro Lys Phe Ser Asp Ile Pro Lys Met 85 90 95
- Ala Glu Met Pro Lys Leu Ser Asp Met Pro Arg Met Ala Asp Ile Pro 100 105 110
- Gln Phe Pro Glu Met Pro Arg Met Val Asp Met Pro Gln Phe Pro Glu 115 120 125
- Ile Pro Arg Met Ala Asp Met Arg Arg Phe Pro Glu Met Ser Lys Ile 130 135 140
- Ala Asp Met Pro Lys Phe Pro Asp Met Pro Asn Val Thr Glu Met Pro 145 150 155 160
- Lys Leu Ala Asp Leu Pro Arg Leu Ala Asp Met Pro Ser Ile Ala Asp 165 170 175
- Met Pro Arg Leu Ser Asp Met Pro Ser Ile Ala Asp Met Pro Arg Leu 180 185 190
- Ser Asp Ile Pro Ser Ile Ala Asp Met Pro Arg Leu Ser Asp Met Pro 195 200 205
- Ser Ile Ala Asp Met Pro Lys Phe Ser Ser Asn Arg Val His Gly Gln 210 215 220
- Ser Tyr His Ile Leu Ala Ile Trp Thr Pro Ser Leu Ser Gly Leu Lys 225 230 235 240
- Glu Phe Phe Thr Pro Leu Ser Asp Leu Ile Lys Pro Glu Ala Ala Ser 245 250 255
- Leu Thr Ser Leu Ala Lys Pro Ser Gly Val Phe Leu Arg Thr Leu Leu 260 265 270

Ala

<213<213<2213<2213)> CI	25 NA DXOD:	lasma		ndii							
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			ttg Leu 20							-		96
			cgc Arg									144
			ggg Gly								_	192
			atg Met									240
			aag Lys									288
			gag Glu 100							_	_	336
			cga Arg							_		384
			gct Ala						cg			425

<210> 14

<211> 141

<212> PRT

<213> Toxoplasma gondii

<400> 14

Arg Gly Ile Pro Asp Gln Arg Ser Ser Arg Ser His Thr Gly Val Glu

1 5 10 15

Ser Leu Val Leu Pro Ser Arg Gly Glu Glu Glu Ala Arg Glu Glu Thr
20 25 30

Ser Ala Thr Arg Gln Met Pro Thr Leu Leu Ser Ser Pro Arg Pro Pro 35 40 45

Leu Ala Leu Gly Leu Gly Asp Lys Ser Pro Cys Gly Glu Trp Val Ser 50 55 60

Pro Asn Asp Met Val Ser Ala Leu Ser Leu Trp Glu Ala Gly Glu Ala 65 70 75 80

Trp Gln Phe Lys Thr Ala Lys Ile Leu Asp Ser Phe Glu Gly Glu Thr
85 90 95

Pro Glu Gly Glu Gly Cys Gly Ala Gln Glu Arg Arg Thr Ala Ala Cys
100 105 110

Lys Leu Val Arg Leu Pro Val Asn Val Glu Gly Arg Ser Thr Lys Val 115 120 125

Trp Ser Leu Ala Leu Leu Ser Ser Leu Arg Leu Lys Ile 130 135 140

<210> 15

<211> 417

<212> DNA

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<220>

<221> CDS

<222> (1)..(417)

<400> 15

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Arg Gly Leu Ser Asp Asp Ala Ser His Ala Glu Thr Pro Ser Pro Leu
1 5 10 15

										gga Gly						96
										acg Thr						144
										tct Ser				_		192
										gcc Ala 75		_		_		240
										tcg Ser				_		288
										cat His						336
										acg Thr						384
					cag Gln		-		_							417
<211 <212)> 16 l> 13 2> PF B> To	89 RT	.asma	ı gor	ndii											
)> 16															
Arg 1	Gly	Leu	Ser	Asp 5	Asp	Ala	Ser	His	Ala 10	Glu	Thr	Pro	Ser	Pro 15	Leu	
Thr	Pro	Ser	Arg 20	Val	Asp	Ser	Phe	Ser 25	Asp	Gly	Val	Glu	Arg 30	Thr	Arg	
Arg	Ser	Ser 35	Pro	Arg	Val	Glu	Glu 40	His	Gln	Thr	Ser	Ser 45	Arg	Glu	Glu	

Lys Ala Ala Thr Glu Arg Val Pro Lys Leu Ser Arg Leu Pro Ser Leu 50 60

Arg Ala Pro Leu Arg Ser Thr Asp Arg Arg Ala Ser Pro Pro Arg Arg 65 70 75 80

Leu Ser Gln Leu Leu Arg Cys Cys Thr Thr Ser Arg Phe Ala Ser Lys
85 90 95

Gly Thr Ala Tyr Pro Asp Glu Glu Trp Gly His Arg Val Arg Ala Gln 100 105 110

Arg Thr Glu Glu Thr Val Ser Ser Leu Thr Thr Lys Arg Leu Leu Thr
115 120 125

Arg Ser Pro Asn Ser Gln Thr Ala Phe Pro Arg 130 135

<210> 17

<211> 507

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(153)

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ggc agg gga agt gga cga cac ccg tcg ctg agc ttt cgc ctg gag tgg 48 Gly Arg Gly Ser Gly Arg His Pro Ser Leu Ser Phe Arg Leu Glu Trp 1 5 10 15

aga cat cta cct gtg agt gaa cca ggc gtt ctg ctt tcg ccg ctc ctt 96
Arg His Leu Pro Val Ser Glu Pro Gly Val Leu Leu Ser Pro Leu Leu
20 25 30

tgc agg cca gag gac aat gat aca aat ata agt gac act ctt ctc ttc 144 Cys Arg Pro Glu Asp Asn Asp Thr Asn Ile Ser Asp Thr Leu Leu Phe 35 40 45

gat atc ggt taactgacaa agaaccacag cggagttaaa atagcagcgt 193
Asp Ile Gly
50

ttgcagttca acgcatgcac aaactgctta actcccacat gcttgccttt gagagacgcg 253

acagcacatc gttcgagctt gcacgcagcg aagacatcta gacagcaatt aggagatgcc 313 tgccgaattt gtatgtaagg cgcaaacgtc tcctcggtgc gaatcacaat tacqcacatt 373 tgcccggact tacatctgtc ttctactggg gtctttcctt gtcaaaccgt gccgctgcaa 433 ctccaaacta gctcgttagt gagatgctgg caaggttttg acaagaatcg agttctgcga 493 ctgcatcgtg gtcg 507 <210> 18 <211> 51 <212> PRT <213> Toxoplasma gondii <400> 18 Gly Arg Gly Ser Gly Arg His Pro Ser Leu Ser Phe Arg Leu Glu Trp 10 Arg His Leu Pro Val Ser Glu Pro Gly Val Leu Leu Ser Pro Leu Leu 20 25 30 Cys Arg Pro Glu Asp Asn Asp Thr Asn Ile Ser Asp Thr Leu Leu Phe 40 35 45 Asp Ile Gly 50 <210> 19 <211> 718 <212> DNA <213> Toxoplasma gondii <220> <221> CDS <222> (1)..(297) <400> 19 gaa ttc cga ctg aat gac tac ctc ttt cag gtg cca gag ggt ccc ccc 48 Glu Phe Arg Leu Asn Asp Tyr Leu Phe Gln Val Pro Glu Gly Pro Pro 1 5 10 15 gcg aga agc cat ggg ttc gac aga aga cga gca gcg agc aaa aac 96 Ala Arg Ser His Gly Phe Asp Arg Arg Ala Ala Ala Ser Lys Asn 20 25

			gaa Glu													144
			ccg Pro													192
			gaa Glu													240
			gat Asp													288
	gga Gly		tgaa	aaaa	igc c	gaag	atga	ıc aç	gcċa	gagt	: aag	jacga	gga			337
ggtg	cago	ac a	agga	itgtc	t ct	tatt	cacc	gag	tctc	gtt	aacc	agcg	tt g	gtct	tatca	397
agag	gtgc	ag ç	gacac	agat	g ag	acat	ccgç	ttc	gtcc	aaa	gaco	agtt	gg a	gcac	tcgag	457
agag	gcaa	iga c	cagaa	igctg	ja gg	gttc	gcga	caç	acat	cca	gctg	rcctc	cg c	gggc	gttgt	517
tcac	tgag	ıga c	ettgg	ıtcgg	ja aa	gggg	agaç	g aaa	cata	gaa	acga	agaa	ca c	caag	acctg	577
gaag	aggt	gc a	gatt	cctc	t tg	ıggca	ctcg	, caç	ıgaga	cgc	cttc	gtca	gt t	tttt	ttgtt	637
cact	caac	gg a	ectct	gtcg	ıt ca	cgag	ıggaa	cto	agac	aga	gacc	tcaa	gg a	gaca	gagga	697
acgo	aacç	jca c	egteg	gaat	t c											718

<210> 20

<211> 99

<212> PRT

<213> Toxoplasma gondii

<400> 20

Glu Phe Arg Leu Asn Asp Tyr Leu Phe Gln Val Pro Glu Gly Pro Pro 1 5 10 15

Ala Arg Ser His Gly Phe Asp Arg Arg Arg Ala Ala Ala Ser Lys Asn 20 25 30

Ala Thr Glu Glu Thr Arg Arg Leu Ala Gly Lys Glu Thr Pro Pro His 35 40 45

Arg Glu Ala Pro Glu Lys Thr Thr Arg Gly Glu Glu Asp Arg Gln Glu Ser Glu Arg Glu Arg Arg Arg Ala Gly Val Met Asp Lys Lys Asn Gln 70 75 Asp Leu Asp Asp Glu Thr Arg Arg Gly Thr Ala Glu Glu Glu Arg 90 Asn Gly Asp <210> 21 <211> 441 <212> DNA <213> Toxoplasma gondii <220> <221> CDS <222> (1)..(441) <400> 21 cgg atc gcc tcg gca ctt cct cat tat ccg tcg cat ggg cat ttc ctg Arg Ile Ala Ser Ala Leu Pro His Tyr Pro Ser His Gly His Phe Leu 1 gaa gag gaa caa att ttg ctg ttg gat tgg cag tat caa ctt ggg caa 96 Glu Glu Glu Gln Ile Leu Leu Asp Trp Gln Tyr Gln Leu Gly Gln 20 25 cga qgc atg gag tcc ggt gta ccc ccc tgc gtg cag cat ggg gat gcg 144 Arg Gly Met Glu Ser Gly Val Pro Pro Cys Val Gln His Gly Asp Ala 35 40 45 acq aga agt ttg act tca ccg aaa agg gat gtc agt cat gac ggt cac Thr Arg Ser Leu Thr Ser Pro Lys Arg Asp Val Ser His Asp Gly His 55 50 caa gga aac agc gga aca aac gca gat gaa gcc ggc caa ggg gcc atg 240 Gln Gly Asn Ser Gly Thr Asn Ala Asp Glu Ala Gly Gln Gly Ala Met 75 65 70 gca ggc cga gga aag tgc gag tgg agc cgc acc acc ggt gcc aac gta 288 Ala Gly Arg Gly Lys Cys Glu Trp Ser Arg Thr Thr Gly Ala Asn Val

90

85

acg aac aag tee tea gge gte gee agt ttg gag gea eea geg teg tte Thr Asn Lys Ser Ser Gly Val Ala Ser Leu Glu Ala Pro Ala Ser Phe 20 25 144 Ala Gln Glu Gly Asp Gly Gly Arg Arg Glu Glu Ala Ser Gln Ala Lys 40. atg ggg acg tct ccc ccg tcg aat cag gtg atc aac gtt gta gac gaa 192 Met Gly Thr Ser Pro Pro Ser Asn Gln Val Ile Asn Val Val Asp Glu 50 55 gac gag gag gac gag gaa gca gag gcg cta gag gct ccc gg 236 Asp Glu Glu Asp Asp Glu Glu Ala Glu Ala Leu Glu Ala Pro 65 70 <210> 128 <211> 78 <212> PRT <213> Toxoplasma gondii <400> 128 Arg Gly Glu Gly Glu Thr Glu Arg Gly Gln Asn Glu Glu Thr His Ala 10 Thr Asn Lys Ser Ser Gly Val Ala Ser Leu Glu Ala Pro Ala Ser Phe 20 Ala Gln Glu Gly Asp Gly Gly Arg Arg Glu Glu Ala Ser Gln Ala Lys 35 40 Met Gly Thr Ser Pro Pro Ser Asn Gln Val Ile Asn Val Val Asp Glu 50 55 Asp Glu Glu Asp Asp Glu Glu Ala Glu Ala Leu Glu Ala Pro 65 70 75 <210> 129 <211> 569 <212> DNA <213> Toxoplasma gondii <220> <221> CDS <222> (1)..(567)

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		-		-	-				-		-	Gly ggg	-			96
-	-			-			-	_				cca Pro 45	_		-	144
-		_	-	-	_			-			-	tcc Ser				192
												gca Ala				240
_		_	_									gaa Glu				288
		_	_	-								tta Leu				336
		_	_	_		-	_		_		_	gga Gly 125		-		384
	_		_		_	-		-	-			agt Ser		-	_	432
_				-	-	-	-			-		tac Tyr			-	480
	, ,		_	_				-	-	His		gtg Val	-			528
		gta Val										atc Ile	cg			569

180 185

<210> 130

<211> 189

<212> PRT

<213> Toxoplasma gondii

<400> 130

Arg Ser Arg Phe Gly Pro Glu Gln Phe Ala Ile Ser Asp Val Ser Gly
1 5 10 15

Thr Leu Val Asn Ala Ser Trp Leu Gly Ala Ser Ala Gly Glu Thr Ile 20 25 30

Ala Asp Ser Arg Ala Leu Arg Arg Asp Leu Ser Phe Pro Leu Ser Ser 35 40 45

Arg Gln Leu Arg Glu Arg Gly Leu Ala Ser Gln Asp Ser Ser Leu Ser 50 55 60

Ser Thr Pro Lys Leu Ser Leu Gln His Asp His Phe Ala Lys Thr Leu 65 70 75 80

Val Lys Arg Arg Ala Leu Ser Ala Thr Asn Ser Thr Glu Arg Ser Gly
85 90 95

Lys Pro Val Arg Cys Phe Thr Glu Thr Ser Val Arg Leu Gly Ala Pro 100 105 110

Thr Gln Pro Val Met Glu Glu Met Pro Leu Gly Glu Gly Glu Val Asn 115 120 125

Leu Val Ser Glu His Asp Asp Tyr Ala Glu Ser Thr Ser His Leu Asp 130 135 140

Thr Val Asn Gly Arg Glu Arg Arg Glu Glu Arg His Tyr Ala Glu Thr 145 150 155 160

Glu Ala Thr Asp Glu Phe Lys Ser Ala Met His His Val Thr Ser Pro 165 170 175

Gly Gly Val Pro Ala Thr Lys Lys Val Val Trp Lys Ile 180 185

<210> 131

<211> 232

<212> DNA

<213> Toxoplasma gondii

<400> 131

cggcgactca gatgggagtg agaaagatgc aaacaggtgc tgaaaaaaca ccacttaata 60 gaggagacaa accccggtgg agaaagcgaa acgagactgg aacggcaacg aaatagagaa 120 gacacagccc caaactcccg acagcgtgtt gctctgtcgg gcaggcaggc caagctggca 180

agccgctage atgccacgtg ctgtactgct ggcccgaaac tacagtgcgc ac 232

<210> 132

<211> 276

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(276)

<400> 132

ccc gga att ccg gct ccg ggt cgc aaa gcg atc cat ttg ata aaa gac 48
Pro Gly Ile Pro Ala Pro Gly Arg Lys Ala Ile His Leu Ile Lys Asp
1 5 10 15

tgc gtt ttc tgc ctt ggg gaa ctc ttc ttg aat ggc acg aga ggc cac 96 Cys Val Phe Cys Leu Gly Glu Leu Phe Leu Asn Gly Thr Arg Gly His 20 25 30

gcg ccc aga ata cag gca gcc tct ccg aag tca ctc acc ttg tac gat 192
Ala Pro Arg Ile Gln Ala Ala Ser Pro Lys Ser Leu Thr Leu Tyr Asp
50 55 60

ctt gtg cac agt gat gta ggg cgc atg cag aac gac gcc tcc aac atg 240 Leu Val His Ser Asp Val Gly Arg Met Gln Asn Asp Ala Ser Asn Met 65 70 75 80

aat att ctc ctc ggc caa ggc cgc cgc caa gta gcg 276 Asn Ile Leu Gly Gln Gly Arg Arg Gln Val Ala 85 90

<210> 133

<211> 92

<212> PRT

<213> Toxoplasma gondii

<400> 133

Pro Gly Ile Pro Ala Pro Gly Arg Lys Ala Ile His Leu Ile Lys Asp 1 5 10 15

Cys Val Phe Cys Leu Gly Glu Leu Phe Leu Asn Gly Thr Arg Gly His 20 25 30

Arg Gln Arg Glu Arg Glu Gly Lys Pro Lys Lys Gln Thr Gly Ser Glu 35 40 45

Ala Pro Arg Ile Gln Ala Ala Ser Pro Lys Ser Leu Thr Leu Tyr Asp 50 55 60

Leu Val His Ser Asp Val Gly Arg Met Gln Asn Asp Ala Ser Asn Met 65 70 75 80

Asn Ile Leu Leu Gly Gln Gly Arg Arg Gln Val Ala 85 90

<210> 134

<211> 309

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(309)

<400> 134

cgc gga cac act gga gag agg tgg tcg gac agg gag gga gaa tcc gag 48
Arg Gly His Thr Gly Glu Arg Trp Ser Asp Arg Glu Gly Glu Ser Glu
1 5 10 15

atg tgc agt gga gga caa atg gaa aag aga gag agc cga cgc gtt tct 96
Met Cys Ser Gly Gly Gln Met Glu Lys Arg Glu Ser Arg Arg Val Ser
20 25 30

ttt gcg gat gaa gag atg cgg aat ccg aca gaa aac ctg aag gta gat 144
Phe Ala Asp Glu Glu Met Arg Asn Pro Thr Glu Asn Leu Lys Val Asp
35 40 45

gcc aac tgt gtg.ctc gaa ggt ctg tcc acc tca gtg tgt gcg agg cgg 192

Ala Asn Cys Val Leu Glu Gly Leu Ser Thr Ser Val Cys Ala Arg Arg 50 55 60

ctg aag agg caa aag cga act gca ggt cag tct ggc ttc ctc gca ata 240 Leu Lys Arg Gln Lys Arg Thr Ala Gly Gln Ser Gly Phe Leu Ala Ile 65 70 75 80

cga aac gtc caa ggc acc gcg acc gcc cta aaa cac cct gat tcc aca 288 Arg Asn Val Gln Gly Thr Ala Thr Ala Leu Lys His Pro Asp Ser Thr 85 90 95

gga cga cgg tct tgg gat ccg

Gly Arg Arg Ser Trp Asp Pro

100

<210> 135

<211> 103

<212> PRT

<213> Toxoplasma gondii

<400> 135

Arg Gly His Thr Gly Glu Arg Trp Ser Asp Arg Glu Gly Glu Ser Glu

1 5 10 15

Met Cys Ser Gly Gly Gln Met Glu Lys Arg Glu Ser Arg Arg Val Ser 20 25 30

Phe Ala Asp Glu Glu Met Arg Asn Pro Thr Glu Asn Leu Lys Val Asp 35 40 45

Ala Asn Cys Val Leu Glu Gly Leu Ser Thr Ser Val Cys Ala Arg Arg 50 55 60

Leu Lys Arg Gln Lys Arg Thr Ala Gly Gln Ser Gly Phe Leu Ala Ile 65 70 75 80

Arg Asn Val Gln Gly Thr Ala Thr Ala Leu Lys His Pro Asp Ser Thr 85 90 95

Gly Arg Arg Ser Trp Asp Pro 100

<210> 136

<211> 534

<212> DNA

<213> Toxoplasma gondii

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165 170 175

gtg ctg 534 Val Leu

<210> 137

<211> 178

<212> PRT

<213> Toxoplasma gondii

<400> 137

Arg Ile Glu Ala Glu Ile Ala Arg Gln Lys Glu Arg Glu Ala Lys Leu 1 5 10 15

Arg Arg Arg Leu Ala Ala Val Val Ala Ser Met Leu Val Ala Ala Ser 20 25 30

Leu Tyr Gly Leu Asn Ser Phe Leu His Gly Ser Asp Lys Glu Ile Ser 35 40 45

Ser Met Pro Ser Ser Ile Asp Lys Lys Pro Asp Ser Pro Phe Ala Ala 50 55 60

Gln Leu Gly Thr Ser Leu Glu Ser Glu Ile Gly Ile Pro Glu Glu Lys
65 70 75 80

Ala Ile Pro Glu Ala Ala Asp Ile Ser Ser Phe Ile Glu Asn Leu Ser 85 90 95

Ala Thr Val Ala Gly Asn Ser Val Gln Ala Gln Ser Ile Gly Phe Val 100 105 110

Leu Thr Val Val Val Leu Gly Leu Val Ala Phe Ser Leu Lys Ala Ala
115 120 125

Arg Arg Ser Ser Pro Arg Glu Glu Gln Ala Phe Ser Leu Pro Ala His 130 135 140

Pro Pro Arg Glu Glu Lys Ser Lys Tyr Leu Leu Lys Pro Pro Gln Gln 145 150 155

Pro Lys Pro Arg Arg Leu Lys Arg Gln Leu Arg Lys Tyr Arg Gln Arg 165 170 175

Val Leu

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<210> 138
<211> 423
<212> DNA
<213> Toxoplasma gondii
<220>
\langle 223 \rangle At locations 6, 23 and 34, N = unknown
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gtccggtgga ggatgttgtt gagcccccgt cgggagtgga agacctgccg cagccagagg 120
cagaggegea agtacegace aagggtgttg accatgeege gtegggaggg gaggacateg 180
tggagccaga ggcagagccg cagggactgg tggctggcgc tggtgaggcc gcatcgggag 240
gggaggacct gctagagcca ggggcagcgc cgcagggtcc ggtgaaggat gttgatgagg 300
cggcgtcggg agaggaagaa ctgctggagc cagaggcaaa gccgcagggt tcggtggagg 360
atgttgatga ggcagcgtcg ggaggggagg acctgctaga gccagaggca gaggcgcaag 420
tcc
                                                                    423
<210> 139
<211> 327
<212> DNA
<213> Toxoplasma gondii
<220>
<221> CDS
<222> (1)..(327)
<400> 139
cgc tct caa tca aca aag cca ccc gcg cct tca gac gta gag gac aca
                                                                    48
Arg Ser Gln Ser Thr Lys Pro Pro Ala Pro Ser Asp Val Glu Asp Thr
                                      10
                                                           15
gge tet tet gae aac eeg ggt gae aat gtg aca gag gae aca act gag
                                                                    96
Gly Ser Ser Asp Asn Pro Gly Asp Asn Val Thr Glu Asp Thr Thr Glu
             20
                                  25
                                                       30
agt cca tca cag ggc acc gac ggt tca gca tcc gga ccc ggg tcg act
                                                                    144
Ser Pro Ser Gln Gly Thr Asp Gly Ser Ala Ser Gly Pro Gly Ser Thr
         35
                              40
```

192

240

288

327

cat ccg gaa aac gac gcg ggg gaa cat gag gat ggc gcg tca ctg ggg His Pro Glu Asn Asp Ala Gly Glu His Glu Asp Gly Ala Ser Leu Gly 50 55 caa gac cag caa gag cgc atg gat aaa tct tcc cta ggc aaa gaa aca Gln Asp Gln Gln Glu Arg Met Asp Lys Ser Ser Leu Gly Lys Glu Thr ccc atg ctc gat cag gga aat tcg tca cca gca aca acg ggg tcc ggt Pro Met Leu Asp Gln Gly Asn Ser Ser Pro Ala Thr Thr Gly Ser Gly 85 gcc cat gaa aaa aac gag agc gtg tca gga gtt cca gcg Ala His Glu Lys Asn Glu Ser Val Ser Gly Val Pro Ala 100 105 <210> 140 <211> 109 <212> PRT <213> Toxoplasma gondii <400> 140 Arg Ser Gln Ser Thr Lys Pro Pro Ala Pro Ser Asp Val Glu Asp Thr 5 10 Gly Ser Ser Asp Asn Pro Gly Asp Asn Val Thr Glu Asp Thr Thr Glu 20 25 30 Ser Pro Ser Gln Gly Thr Asp Gly Ser Ala Ser Gly Pro Gly Ser Thr 35 40 His Pro Glu Asn Asp Ala Gly Glu His Glu Asp Gly Ala Ser Leu Gly 50 55 Gln Asp Gln Gln Glu Arg Met Asp Lys Ser Ser Leu Gly Lys Glu Thr

Ala His Glu Lys Asn Glu Ser Val Ser Gly Val Pro Ala 100

85

<210> 141 <211> 444

Pro Met Leu Asp Gln Gly Asn Ser Ser Pro Ala Thr Thr Gly Ser Gly

105

75

<212> DNA <213> Toxoplasma gondii

<220> <221> CDS <222> (1)..(444)

<400> 141

ccg gcg cga act ggc gac gcg cag cct gag ggc aga gag ggg cac agc 48
Pro Ala Arg Thr Gly Asp Ala Gln Pro Glu Gly Arg Glu Gly His Ser
1 5 10 15

cca ctg gaa gac gaa ggg aga gat gcg ttt gga aga cgc gct gcg gaa 96 Pro Leu Glu Asp Glu Gly Arg Asp Ala Phe Gly Arg Arg Ala Ala Glu 20 25 30

gac gag aga aac aga gga aat ccg aat gcg gct ggc gag act tcc caa 144 Asp Glu Arg Asn Arg Gly Asn Pro Asn Ala Ala Gly Glu Thr Ser Gln 35 40 45

gac gag gca gag aac gcg caa gcg tcc ctg cgg ttc gct gcg aga gag 192 Asp Glu Ala Glu Asn Ala Gln Ala Ser Leu Arg Phe Ala Ala Arg Glu 50 55 60

aaa cct ctc gaa gtc ctc aga ttc cga gaa gac act gca gac act ctg 240 Lys Pro Leu Glu Val Leu Arg Phe Arg Glu Asp Thr Ala Asp Thr Leu 65 70 75 80

acg tat gca gac tat cca aac agc gtg gag ttc aca ccc gca gac atg 288
Thr Tyr Ala Asp Tyr Pro Asn Ser Val Glu Phe Thr Pro Ala Asp Met
85 90 95

tgc gcc tac tca tgc tgg ctg acc tcg cgc ttc aac cca gac aac cca 384
Cys Ala Tyr Ser Cys Trp Leu Thr Ser Arg Phe Asn Pro Asp Asn Pro
115 120 125

aac agc cac tgt gga aaa gga aaa aac gag aaa cgc cga ttc gac gac 432 Asn Ser His Cys Gly Lys Gly Lys Asn Glu Lys Arg Arg Phe Asp Asp 130 135 140

gac tac gat ccg
Asp Tyr Asp Pro

145

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48

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Glu Glu Asp

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PCT/US98/27137

WO 99/32633

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	2> Di															
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<220)>															
	l> CI	os														
	_	-	(288))												
	,	·	, ,													
< 400)> 26	65														
			gct													48
Arg	Asp	Pro	Ala	Ala	Pro	Asn	Ser	Thr	Gln	Ala	Val	Ala	Ala	Ala	Arg	
1				5					10					15		
200	ata	at a	at a	ata	222	200	a 20	~~~	~	~ + ~						0.0
			gtg Val													96
1	vuı	vai	20	riec	цуз	1111	ASP	25	GIU	vaı	ser	GIA		Asn	Leu	
			20					23					30			
agt	cag	ccg	ggt	agg	cqt	ccq	ccq	tca	cca	aaσ	cca	caa	acα	acq	aag	144
			Gly													111
		35	_	_			40			4 -		45			2,0	
			aga													192
Phe		Arg	Arg	Glu	Ser	Pro	Asp	Arg	Arg	Gly	Thr	Arg	Arg	Arg	Thr	
	50					55					60					
			ggc													240
	ser	Arg	Gly	АТА		Ser	Arg	Val	Trp		Gly	Glu	Asn	Gln	_	
65					70					75					80	
aσa	caa	tct	acc	atc	gac	gat	tea	ata	cca	act	aac	CCC	ato	act	++~	200
aga	cgg	tct	gcc	gtc	gac	gat	tcg	ata	ccg	gct	aac	ccc	atc	act	tta	288

Arg Arg Ser Al.: Val Asp Asp Ser Ile Pro Ala Asn Pro Ile Ala Leu • 85 90

aacgcgtggc :: nut gcgat ccg

311

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<211> 96

<212> PRT

<213> Toxe; .usra usndii

<400> 200

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Thr Val Val ... M: Lys Thr Asp Ala Glu Val Ser Gly Asp Asn Leu

Ser Gln I::: Arg Pro Pro Ser Pro Lys Pro Gln Thr Thr Lys 40

Phe Pro A: . .: . . . Ser Pro Asp Arg Gly Thr Arg Arg Arg Thr 55

Glu Ser A:: Ser Arg Val Trp Pro Gly Glu Asn Gln Arg 75

Arg Arg : Asp Asp Ser Ile Pro Ala Asn Pro Ile Ala Leu 90

<210> 2e

<211> 30 · ·

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<213> 1-1111

<220>

<221> C:

<222> (.

<400> 2.

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ctc tcc ·· : ago gto ctg ctt gtt ctc gaa ccc gca gag ccc Leu Ser : . : : Ser Val Leu Leu Val Leu Glu Pro Ala Glu Pro 25 30

ctg cta tcc tct tgg ccc cac ccg ggg aga aga gac act ttt ctt gaa 144 Leu Leu Ser Ser Trp Pro His Pro Gly Arg Arg Asp Thr Phe Leu Glu 35 40 ggc gat ggc gcg ggc atc ccg tct cct tca tct cgg ccg agt cgc gcg 192 Gly Asp Gly Ala Gly Ile Pro Ser Pro Ser Ser Arg Pro Ser Arg Ala 50 55 gcc gac cat tac acg aga ctc tcc acg att cgg tct ctt gcc agg gat 240 Ala Asp His Tyr Thr Arg Leu Ser Thr Ile Arg Ser Leu Ala Arg Asp 70 gga gag gtc gac tcc gag ctg gcg ggg gga ccg cag gaa aga gaa agt 288 Gly Glu Val Asp Ser Glu Leu Ala Gly Gly Pro Gln Glu Arg Glu Ser 85 gtc aga gtg gat ccg 303 Val Arg Val Asp Pro 100 <210> 268 <211> 101 <212> PRT <213> Toxoplasma gondii <400> 268 Asp Glu Ala Leu Pro Leu Phe Gly Ala Asn Asp Gly Thr Ser Val Arg 10 Leu Ser Leu Asp Arg Ser Val Leu Leu Val Leu Glu Pro Ala Glu Pro 20 25 Leu Leu Ser Ser Trp Pro His Pro Gly Arg Arg Asp Thr Phe Leu Glu 40 Gly Asp Gly Ala Gly Ile Pro Ser Pro Ser Ser Arg Pro Ser Arg Ala 50 55 Ala Asp His Tyr Thr Arg Leu Ser Thr Ile Arg Ser Leu Ala Arg Asp 65 70 80

Gly Glu Val Asp Ser Glu Leu Ala Gly Gly Pro Gln Glu Arg Glu Ser

90

85

Val Arg Val Asp Pro

100

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Ala Gln Glu Gly Asp Gly Gly Arg Glu Glu Ala Ser Gln Ala Lys

40

35

Met Gly Thr Ser Ser Pro Ser Asn Gln Val Ile Asn Val Val Asp Glu 50 55 60

Asp Glu Gl. Asp Asp Glu Glu Ala Glu Ala Gln Glu Ala Pro 65 70 75

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<211> 42 ·

<212> DNA

gondii دهند، ; Tux و gondii

<220>

<221> CL.

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Arg Gl, I. 4: Asp Gln Arg Ser Ser Arg Ser His Thr Gly Val Glu

1 5 10 15

tet qc, ..: cag atg eeg acg ett ete tet teg eeg agg eet eea 144 Ser Ala ::: `:: Gln Met Pro Thr Leu Leu Ser Ser Pro Arg Pro Pro 40 45

ctc g.: ::: ttg gga gac gag tct ccc tgc gga gag tgg gtg tcg 192 Leu A.:: . !eu Gly Asp Glu Ser Pro Cys Gly Glu Trp Val Ser

ccg a... : 1't tct gcg ttg tcc ctc tgg gaa gca ggc gag gct 240

Pro A · · · · ... Ser Ala Leu Ser Leu Trp Glu Ala Gly Glu Ala

65 70 75 80

tgg Ca: ... gcg aaa att ctt gac tct ttc gaa ggg gag acc 288
Trp G:: T:r Ala Lys Ile Leu Asp Ser Phe Glu Gly Glu Thr
-5 90 95

CCa Ga to tgc ggc gca cag gaa aag gac agc cgc atg caa 336
Pro Gl. Cys Gly Ala Gln Glu Lys Asp Ser Arg Met Gln
105 110

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<210> 272

<211> 141

<212> PRT

<213> Toxoplasma gondii

<400> 272

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Ser Ala Thr Arg Gln Met Pro Thr Leu Leu Ser Ser Pro Arg Pro Pro 35 40 45

Leu Ala Leu Gly Leu Gly Asp Glu Ser Pro Cys Gly Glu Trp Val Ser 50 55 60

Pro Asn Asp Met Val Ser Ala Leu Ser Leu Trp Glu Ala Gly Glu Ala 65 70 75 80

Trp Gln Phe Lys Thr Ala Lys Ile Leu Asp Ser Phe Glu Gly Glu Thr
85 90 95

Pro Glu Gly Glu Cys Gly Ala Gln Glu Lys Asp Ser Arg Met Gln
100 105 110

Ala Gly Ala Thr Pro Gly Glu Arg Gly Gly Ala Val Asp Glu Gly Val 115 120 125

Glu Leu Gly Ser Ser Phe Phe Ser Ala Ser Glu Asp Pro 130 135 140

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ctg Leu	cct Pro	cct Pro	atg Met 20	cca Pro	ctg Leu	ccc Pro	gaa Glu	gca Ala 25	ccc Pro	gaa Glu	gac Asp	ttt Phe	gac Asp 30	cag Gln	gct Ala	96
					gag Glu											144
cca Pro	ctg Leu 50	ccc Pro	gag Glu	gca Ala	ccc Pro	gaa Glu 55	gac Asp	ttt Phe	gac Asp	cag Gln	cct Pro 60	cct Pro	atg Met	cca Pro	ctg Leu	192
ccc Pro 65	gag Glu	gca Ala	ccc Pro	gaa Glu	gac Asp 70	ttt Phe	gac Asp	cag Gln	gct Ala	cct Pro 75	atg Met	cca Pro	ctg Leu	ccc Pro	gaa Glu 80	240
gca Ala	ccc Pro	gaa Glu	gtc Val	ttt Phe 85	gac Asp	cag Gln	gct Ala	cct Pro	atg Met 90	cca Pro	ctg Leu	ccc Pro	gag Glu	gca Ala 95	ccc Pro	288
gaa Glu	gtc Val	ttt Phe	gac Asp 100	cag Gln	gct Ala	cct Pro	atg Met	cca Pro 105	ctg Leu	ccc Pro	gaa Glu	gca Ala	ccc Pro 110	gaa Glu	gac Asp	336
ttt Phe	gac Asp	cag Gln 115	gct Ala	cct Pro	atg Met	cca Pro	ctg Leu 120	ccc Pro	gaa Glu	gca Ala	ccc Pro	gaa Glu 125	gtc Val	ttt Phe	gac Asp	384
cag Gln	gct Ala 130	cct Pro	atg Met	cca Pro	ctg Leu	ccc Pro 135	gag Glu	gca Ala	ccc Pro	gaa Glu	gac Asp 140	ttt Phe	gac Asp	cag Gln	gct Ala	432
ect Pro 145	atg Met	cca Pro	gtg Val	ccc Pro	gag Glu 150	gca Ala	ccc Pro	gaa Glu	gac Asp	ttt Phe 155	gac Asp	cag Gln	gct Ala	cct Pro	gag Glu 160	480
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<211> 171

<212> PRT

<213> Toxoplasma gondii

<400> 274

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Leu Pro Pro Met Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala 20 25 30

Pro Met Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala Pro Met 35 40 45

Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Pro Pro Met Pro Leu 50 55 60

Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala Pro Met Pro Leu Pro Glu 65 70 75 80

Ala Pro Glu Val Phe Asp Gln Ala Pro Met Pro Leu Pro Glu Ala Pro 85 90 95

Glu Val Phe Asp Gln Ala Pro Met Pro Leu Pro Glu Ala Pro Glu Asp 100 105 110

Phe Asp Gln Ala Pro Met Pro Leu Pro Glu Ala Pro Glu Val Phe Asp 115 120 125

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Pro Leu Pro Glu Ala Ala Glu Glu Phe Asp Pro 165 170

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WO 99/32633			PCT/US98/2713
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Gly Arg Val Ser Gln Lys Lys Thr Leu Val Cys Ala Arg Arg Arg Gln
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                                      10
                                                           15
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97

1225

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<212> PRT

<213> Toxoplasma gondii

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<400> 283

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Ser Leu Arg Pro Leu Gly Arg Thr Glu Phe Ser Arg
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<210> 284

<211> 1225

<212> DNA

<213> Toxoplasma gondii

<400> 284

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WO 99/32633	PCT/US98/27137
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WO 99/32633
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     Franci
<400 . . . - .
tttccca;; ":; tqtc
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<210 - 2 +
<211> 1
<212> 1***
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    : : -
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tcatt:.
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<210> 293

<211> 73

<212> PRT

<213> Toxoplasma gondii

<400> 293

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20 25 30

Pro Ala Ala Asp Cys Ala Phe His Arg Gly Glu Gly Arg Gln Gly
35 45

Ser Gly Val Leu Gly Gln Val Ala Ser Gly Ala Ser Ala Pro Val Gly 50 55 60

Val Arg Gly Arg Arg Val Ser Val 65 70

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- <213> Artificial Sequence
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- <400> 296

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- <210> 297
- <211> 19

VO 99/32633	PCT/US98/27137
212> DNA	
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Primer	
<400> 299	
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gcgtttcgac agttctatca c
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      Primer
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<211> 9

<212> PRT

<213> Toxoplasma gondii

<400> 307

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1 5

<210> 308

<211> 2417

<212> DNA

<213> Toxoplasma gondii

<400> 308

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<211> 20

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<223> Description of Artificial Sequence: Synthetic Primer

<400> 309

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<210> 310

<211> 18

<212> DNA

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<400> 310

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<210> 311

<211> 1785

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(75)

<400> 311

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Ile Trp Gln Pro Pro Ala His Phe
20 25

acttgtgcag aggagaaaga aggtggccat gattggctct ggcatgattg gtggcactat 155 gggctacctg tgcgctctcc gtgagctcgc tgacgtcgtt ctctacgatg ttgtcaaagg 215 tatgcccgag ggtaaggctc ttgacctgag ccatgtgacc tccgtggtcg acaccaacgt 275 tteegteegt getgagtaet ettaegagge egegeteace ggtgeggaet gegttategt 335 taccgccggt ctgaccaagg tgccgggcaa gcccgactcc gagtggagcc gaaacgatct 395 gctcccgttc aactcgaaga tcattcgcga gatcggtcag aacatcaaga agtactgccc 455 caagaccttc atcatcgtgg tcaccaaccc gctggactgc atggtcaagg tcatgtgcga 515 ggcctctggc gtcccgacca acatgatctg cggtatggcc tgcatgctcg actctggtcg 575 cttccgccga tacgtcgccg acgcgctgtc tgtctctccc cgcgacgtcc aggccaccgt 635 categgeaca caeggegact geatggteec gettgteegg tacattaceg tgaacgacta 695 cccgatccag aagttcatca aggacggcgt agtcacggag aagcagctcg aggagatcgc 755 tgagcacacc aaagtgtctg gcggcgagat cgtccgcttc ctcggccagg gttccgctta 815 ctacgccccc geogeateeg etgtegeeat ggeaacatee ttettgaacg acgaaaageg 875 cgtcatcccg tgcagtgtgt actgcaacgg agagtacggc ttgaaggaca tgttcattgg 935 teteceggee gteattggag gegeeggeat egagegegte ategageteg agetgaacga 995 ggaggagaag aagcagttcc agaagtccgt cgacgacgtc atggcgctca acaaggcggt 1055

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<210> 312

<211> 24

<212> PRT

<213> Toxoplasma gondii

<400> 312

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Ile Trp Gln Pro Pro Ala His Phe 20

<210> 313

<211> 1785

<212> DNA

<213> Toxoplasma gondii

<400> 313

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Primer

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189

atg gcc ttc ccc gag aac tgc cca gag ctc gtg gcc ttc gcc gcc tgc Met Ala Phe Pro Glu Asn Cys Pro Glu Leu Val Ala Phe Ala Ala Cys

50	55	60
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		tgc Cys														336
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		ctg Leu														576
		gtc Val 195														624
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		gaa Glu														720
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	a atc u Ile															864	
	g cat u His 290															912	
	a atc u Ile 5													-		960	
	g cat u His															1008	
	c cct u Pro															1056	
	t cct e Pro															1104	
	t gaa o Glu 370														-	1152	
	t gag o Glu 5															1200	
	t gaa o Glu															1248	
	t cct l Pro															1296	

atc cct gaa gga gag cat gtt cct gaa gag ctc cct gaa ggc gag cat

Ile Pro Glu Gly Glu His Val Pro Glu Glu Leu Pro Glu Gly Glu His

1344

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cat gct cca His Ala Pro	gag gaa gag act Glu Glu Glu Thr 485	c cct gca cct g Pro Ala Pro G 490	ag gag acc gaa aag lu Glu Thr Glu Lys . 495	gag 1488 Glu
			cc ggt ggt gtc gtc la Gly Gly Val Val 510	
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		Glu Asp Glu V	tg atg ttt gag agc al Met Phe Glu Ser 540	
		Glu Asn Arg G	ag agc gag acg gtc lu Ser Glu Thr Val 55	
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Asn Tyr Gly Ala Phe Ser Asp Cys Gly Val Pro Leu Arg Gly Phe Ala 35 40 45

Met Ala Phe Pro Glu Asn Cys Pro Glu Leu Val Ala Phe Ala Ala Cys 50 . 55 60

Asp Ala Pro Ala Pro Pro Gln Glu Asp Arg Cys His Ser Phe Ser Ala 65 70 75 80

Trp Ser Lys Cys Thr His Ile Pro Gly Thr Thr Leu Tyr Glu Gln Thr
85 90 95

Arg Ser Cys Asp Gly Met Asp Leu Thr Glu Ser Arg Phe Cys Thr Pro 100 105 110

Asp Glu Glu Val Gly Ser Asp Val Ser Thr Asp Val Ala Ser Glu Cys
115 120 125

Gly Ser Leu Gly Glu Phe Gly Glu Cys Val Asn Gly Leu Gln Glu Arg 130 135 140

Ser Tyr Ser Asp Cys Pro Asp His Lys Glu Val Arg Gln Cys Ser Asp 145 150 155 160

Glu Ser Cys Ser Ala Phe Gly Glu Trp Ser Pro Cys Gly Glu Pro Gln 165 170 175

Gln Gly Leu Arg Ile Arg Lys Arg Arg Ala Cys Asp Asn Val His Cys 180 185 190

Ala Cys Val Glu Ala Glu Val Cys Gly Asp Val Thr Pro Glu Ile Glu 195 200 205

Glu Glu Glu Gly Glu His Phe Pro Pro Glu Glu Gly Glu Val Leu Pro 210 215 220

- Pro Tyr Glu Glu Gly Pro Gly Glu Gly Glu Leu Val Pro Pro Glu Glu 225 230 235 240
- Glu Ile Pro Glu Gly Glu His Val Pro Glu Glu Glu Ile Pro Glu Gly
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- Glu His Ile Pro Glu Glu Leu Pro Glu Gly Glu His Val Pro Glu Glu 260 265 270
- Glu Ile Pro Glu Gly Glu His Val Pro Glu Glu Glu Ile Pro Glu Gly
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- Pro Glu Gly Glu His Val Pro Glu Glu Glu Ile Pro Glu Gly Glu His
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His Ala Pro Glu Glu Glu Thr Pro Ala Pro Glu Glu Thr Glu Lys Glu
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Gly Val Leu Leu Ile Ala Gly Gly Ala Gly Ala Ala Val Tyr Ala Asn 515 520 525

Gln Gly Gly Val Glu Ala Ala Glu Asp Glu Val Met Phe Glu Ser Glu 530 540

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125

120

11.

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			gga Gly													480
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			gac Asp 180													576
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<211> 197

<212> PRT

<213> Toxoplasma gondii

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190

tacacca::taa aataaaatgg cacgtgatca agaaagagcg gacggacaca 300

aatggctgcg ttcgtctggt acctagetca gagtgtcacg cegeeteece ttegaaggae 360 agcogcagac gotocgcocg taacacagaa tacotogcga toacggtttt gtogcacgcc 420 tgcatgtgcc cacacagact actagtctac tttagtccat gtctgcccat gcgtcatctt 480 cgatctcaat gaccgtctcg ctctcgcggt tctcgccagc ctgggttccg tcttcttcgc 540 tctcaaacat cacttcgtct tcagctgctt caacgccacc ttggtttgcg tacacggcag 600 cacctgcacc accagcaatg agcaacacac ctccgacgac accaccggca atcgctgcga 660 ctggcacgcc ttcttcctcc tccttttcgg tctcctcagg tgcaggagtc tcttcctctg 720 gagcatgttc teetteaggg gteteeteet eaggaacatg etegeettea gggattteet 780 cttcaggaac atgctctcct tcagggatct cctcctcagg aacatgctcg ccttcaggga 840 getetteagg aacatgetet cetteaggga teteeteete aggaataagt teteeteeag 900 ggagctcctc ctcaggaaca tgctcgcctt cagggatctc ctcctcagga acatgctctc 960 cttcagggaa ctcctcctca ggaatatgtt ctccttcagg gatttcctcc tcaggaacat 1020 getegeette agggaactee teaggaatat gtteteette agggatetee tetteaggaa 1080 catgctcgcc ttcagggagc tcctcatgaa tatgctctcc ttcagggaac tcttcatgaa 1140 tatgctctcc ttcagggagc tcctcaggaa tatgttctcc ttcagggaac tcttcaggaa 1200 catgttetee tteagggatt teetetteag gaacatgtte teetteaggg attteeteet 1260 caggaacatg ttcgccttct gggaactctt caggaacatg ctctccttca gggatttcct 1320 cttcaggaac atgttctcct tcagggattt cctcctcagg aacatgctcg ccttctggga 1380 getetteagg aatatgttet cetteaggga ttteeteete aggaacatgt teteetteag 1440 ggateteete etegggagga acaageteae eeteaecagg accetettea tatggaggea 1500 agacctcgcc ttcttcaggg gggaaatgtt cgccttcttc ctcctcaatc tctggggtga 1560 categoegea gaceteggee tegacacagg egeagtgeae gttgtegeat geacgtetet 1620 tgcggatacg caggcettge tggggttece egcagggtga ceaetegeeg aaggeagage 1680 aggattegte agageactga eggaetteet tatgateggg geagteegag tagettetet 1740

PCT/US98/27137 WO 99/32633 cctgaaggcc gttcacacac tcgccgaact cgccgaggga accgcattcg gaagcgacgt 1800 cagtggaaac gtccgagccg acctcctcgt cgggagtgca gaagcgggac tcggtcaaat 1860 ccatgccatc gcaggagcgc gtctgctcgt acagagtagt gccgggtatg tgtgtgcact 1920 tggaccaggc cgagaaagaa tggcagcggt cctcttgggg aggcgcggga gcatcgcagg 1980 cggcgaaggc cacgagctct gggcagttct cggggaaggc catggcgaag ccgcgaagag 2040 gcattccaca gtcggagaag gcaccatagt tgccgcatgc ttgcccagcc tgagttgggc 2100 actccatggt ttcgacttgg ttgacgcacg cctcgctctc ggtgtcttct ctccggcggg 2160 tgctcatgtt gtttccttcg ccgggacacg ggctccacgg acccacggtc cacatgcgtt 2220 gccgtcttcc tcaccaccct cgccggggc gacagtcggg ggctggggca gggagccgac 2280 ttcgcaggtg atcgactcaa ccttgtctgg gaggtccaca cagtagcggc tgctgaggtg 2340 agtctcggga tcgcagcgag tgaatgcact ccagtggttg caagaattcc tg 2392 <210> 332 <211> 21 <212> DNA <213> Artificial Sequence <220> <223> Description of Artificial Sequence: Synthetic Primer <400> 332 21 ggaactgcat ccgttcatga g <210> 333 <211> 19 <212> DNA <213> Artificial Sequence <220> <223> Description of Artificial Sequence: Synthetic Primer <400> 333

19

tcttaaagcg ttcgtggtc

WO 99/32633 PCT/US98/27137 ggg tog to: the tgt gtg gtt gat gcg tgt ttg gcg tct gcg ggt aga 336 Gly Ser Se: ...: Cys Val Val Asp Ala Cys Leu Ala Ser Ala Gly Arg 105 cat cag go: ::: age atg cgt ccg ttt gca cga gat gga ttc ggc gag 384 His Gln Ala Ala Ear Met Arg Pro Phe Ala Arg Asp Gly Phe Gly Glu 111 120 125 tet act $g^{\perp})$ m: a:c aga cec egt egg gae gge etg eea egt tet 432 Ser Thr Ala ... A :. Arg Pro Arg Arg Asp Gly Gly Leu Pro Arg Ser 130 135 ctt gga to: 441 Leu Gly So: 145 <210> 2: <211> 147 <212> PFT <400> 2: Arg Ile /.. . . +: Leu Pro His Tyr Pro Ser His Gly His Phe Leu 10 Glu Glu G: Leu Leu Leu Asp Trp Gln Tyr Gln Leu Gly Gln 25 Arg Gly Mo ...: Gly Val Pro Pro Cys Val Gln His Gly Asp Ala 40 · : Ser Pro Lys Arg Asp Val Ser His Asp Gly His Thr Arg : · · 50 55 Gln Gly F The Asn Ala Asp Glu Ala Gly Gln Gly Ala Met 75 ..0 65 Ala Gly A: ys Glu Trp Ser Arg Thr Thr Gly Ala Asn Val 90 Gly Ser : ... I Val Asp Ala Cys Leu Ala Ser Ala Gly Arg 105 110 : Met Arg Pro Phe Ala Arg Asp Gly Phe Gly Glu His Gln A.

Ser Thr A: .. Arg Pro Arg Arg Asp Gly Gly Leu Pro Arg Ser

120

125

130 135 140

Leu Gly Ser 145

<210> 23

<211> 428

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(426)

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1 5 10 15

gtg gaa aag gat cat ttc ggg agt cgc gag agg cac tcg aat ggg gaa 96 Val Glu Lys Asp His Phe Gly Ser Arg Glu Arg His Ser Asn Gly Glu 20 25 30

gag ttc aag aca cag gga aac gtt ggt cga ggt tca ctg agg cag gaa 144 Glu Phe Lys Thr Gln Gly Asn Val Gly Arg Gly Ser Leu Arg Gln Glu 35 40 45

ccc ttt acc gat gga gtg tac cac gac agg cag cag cgc ttc tcg gag 192
Pro Phe Thr Asp Gly Val Tyr His Asp Arg Gln Gln Arg Phe Ser Glu
50 55 60

aaa gaa cct gcg aag ccg atg ttc act tcc ctc gcg gat ccg agc gtg

Lys Glu Pro Ala Lys Pro Met Phe Thr Ser Leu Ala Asp Pro Ser Val

65 70 75 80

agg aga cat ttt aag gag gaa gaa gaa cga cgg aaa ttc cag gaa aag 288 Arg Arg His Phe Lys Glu Glu Glu Glu Arg Arg Lys Phe Gln Glu Lys 85 90 95

gca gaa gag gag atc ttg cgc ctt ctc aaa cgc gca gct gag tgc agc 336
Ala Glu Glu Ile Leu Arg Leu Leu Lys Arg Ala Ala Glu Cys Ser
100 105 110

gag gaa gat ttg aaa agg gaa gaa cgc tcc gaa aag gct acc gaa aag 384 Glu Glu Asp Leu Lys Arg Glu Glu Arg Ser Glu Lys Ala Thr Glu Lys 115

ggg tcc cgt ctc ttc tct gga gag gag gtg cga ttc ttt ccg cc Gly Ser Arg Leu Phe Ser Gly Glu Glu Val Arg Phe Phe Pro 130 135 140

428

<210> 24

<211> 142

<212> PRT

<213> Toxoplasma gondii

<400> 24

Arg Arg Arg Gln Arg Ala Asp Pro Ser Asp Trp Glu Gly Cys Glu Asn 1 5 10 15

Val Glu Lys Asp His Phe Gly Ser Arg Glu Arg His Ser Asn Gly Glu 20 25 30

Glu Phe Lys Thr Gln Gly Asn Val Gly Arg Gly Ser Leu Arg Gln Glu 35 40 45

Pro Phe Thr Asp Gly Val Tyr His Asp Arg Gln Gin Arg Phe Ser Glu 50 60

Lys Glu Pro Ala Lys Pro Met Phe Thr Ser Leu Ala Asp Pro Ser Val 65 70 75 80

Arg Arg His Phe Lys Glu Glu Glu Glu Arg Arg Lys Phe Gln Glu Lys
85 90 95

Ala Glu Glu Glu Ile Leu Arg Leu Leu Lys Arg Ala Ala Glu Cys Ser 100 105 110

Glu Glu Asp Leu Lys Arg Glu Glu Arg Ser Glu Lys Ala Thr Glu Lys 115 120 125

Gly Ser Arg Leu Phe Ser Gly Glu Glu Val Arg Phe Phe Pro 130 135 140

<210> 25

<211> 282

<212> DNA

<213> Toxoplasma gondii

<400> 25

cgcgacccgc tgccagtgtt ttgagtctaa ccgccgtatg tcgcggattc cacgtggaaa 60

acgacggacc gtcaagacgc ccgagagtgc cgcaatttca cggaccgttc gttcgattcc 120

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tegeggatet attestacaa tgggagaate gtetgatgte tecaetgtee egetacegea 240
                                                             282
caggiccist iningocaca coggiagaca gigagoggog go
<210> 2€
<211> 304
<212> DNA
<213> Texe; andii
<220>
<221> CD:
<222> (: .. ·
<400> 21
cgg act :: . . :: ccg aag cgc agt tcc tcg aaa ccg acg tcg act
                                                             48
Arg Thr .. . Pro Lys Arg Ser Ser Ser Lys Pro Thr Ser Thr
                                  10
tgg gto :: . . . . . . . . . gtc cat act gaa aca aca atg gaa aac gaa ttg
                                                             96
Trp Val : . . . . . Val His Thr Glu Thr Thr Met Glu Asn Glu Leu
                               25
atg aar . . . . . . gac ctc tcg aat gag gct tgg caa aag aaa gaa
                                                             144
Met Ass. .. ... ... Asp Leu Ser Asn Glu Ala Trp Gln Lys Lys Glu
ctt ccc : . . . . aag tgg aca aac agc cct gaa cac tcc ctc ttg
                                                              192
             H: Lys Trp Thr Asn Ser Pro Glu His Ser Leu Leu
                                          60
                        55
     ٦٢
               ... :aa aat agt ctt tca aag cca acc gcg gac tca
                                                              240
aca to:
                :: Glu Asn Ser Leu Ser Lys Pro Thr Ala Asp Ser
Thr Se:
                    70
                                       75
 65
                :: tat ggc aca cgc aga caa agt cac gca aaa gat
                                                              288
cca qu:
               .:: Tyr Gly Thr Arg Arg Gln Ser His Ala Lys Asp
 Pro As;
                                   90
                                                              304
 ctq tt . .
 Leu Ph. ·
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<210> 2

<211> 101

<212> PRT

<213> Toxoplasma gondii

<400> 27

Arg Thr Gly Thr Gly Pro Lys Arg Ser Ser Ser Lys Pro Thr Ser Thr 1 5 10 15

Trp Val Arg Leu Leu Val His Thr Glu Thr Thr Met Glu Asn Glu Leu 20 25 30

Met Asn Gln Val Ser Asp Leu Ser Asn Glu Ala Trp Gln Lys Lys Glu 35 40 45

Leu Pro Val Leu His Lys Trp Thr Asn Ser Pro Glu His Ser Leu Leu 50 55 60

Thr Ser Glu Asp Arg Glu Asn Ser Leu Ser Lys Pro Thr Ala Asp Ser 65 70 75 80

Pro Asp Ser Phe Arg Tyr Gly Thr Arg Arg Gln Ser His Ala Lys Asp 85 90 95

Leu Phe Ser Asp Pro 100

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<211> 284

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(282)

<400> 28

ccg gac ttc ctc atg tct gaa gat gct tgt ctt gtt cgg ttc gtg cga 48
Pro Asp Phe Leu Met Ser Glu Asp Ala Cys Leu Val Arg Phe Val Arg
1 5 10 15

cac gcg tcg gcc aca cac gcg tat aca cgc agg gca agt gcg agg acg 96 His Ala Ser Ala Thr His Ala Tyr Thr Arg Arg Ala Ser Ala Arg Thr 20 25 30

gta aag ccg ctc aaa ggc caa gga gac aaa gaa cag ggt gcg aca gga 144 Val Lys Pro Leu Lys Gly Gln Gly Asp Lys Glu Gln Gly Ala Thr Gly 35 40 45

aga aat gtt gag gca ata aag aag gaa acc cct ctg aga cgg gaa gcg 192 Arg Asn Val Glu Ala Ile Lys Lys Glu Thr Pro Leu Arg Arg Glu Ala 50 55 aga gaa aac gcg ttt ttt tcg acg ttt tcc ccc gac aga gcg agc gcc 240 Arg Glu Asn Ala Phe Phe Ser Thr Phe Ser Pro Asp Arg Ala Ser Ala 65 70 75 80 tcc tgt ctc cgc att cac gcg tgt gcc gcg gca gag gaa ccc gg 284 Ser Cys Leu Arg Ile His Ala Cys Ala Ala Ala Glu Glu Pro 85 <210> 29 <211> 94 <212> PRT <213> Toxoplasma gondii <400> 29 Pro Asp Phe Leu Met Ser Glu Asp Ala Cys Leu Val Arg Phe Val Arg 1 5 10 His Ala Ser Ala Thr His Ala Tyr Thr Arg Arg Ala Ser Ala Arg Thr 20 25 30 Val Lys Pro Leu Lys Gly Gln Gly Asp Lys Glu Gln Gly Ala Thr Gly 35 40 Arg Asn Val Glu Ala Ile Lys Lys Glu Thr Pro Leu Arg Arg Glu Ala 55 Arg Glu Asn Ala Phe Phe Ser Thr Phe Ser Pro Asp Arg Ala Ser Ala 70 75 Ser Cys Leu Arg Ile His Ala Cys Ala Ala Ala Glu Glu Pro 85 <210> 30 <211> 690 <212> DNA <213> Toxoplasma gondii

<220> <221> CDS

<222> (1)..(690)

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185

190

180

ttg cta gag gct gga gcg cag gtt cgt gtg ctt ggg cca acg aca gac 624 Leu Leu Glu Ala Gly Ala Gln Val Arg Val Leu Gly Pro Thr Thr Asp 195 200 ccg gag aca gag acc gct tct cag ctc cag aca act gag ctt gcc acg 672 Pro Glu Thr Glu Thr Ala Ser Gln Leu Gln Thr Thr Glu Leu Ala Thr 210 215 220 690 ctq aca act gtg gat ccg Leu Thr Thr Val Asp Pro 225 230 <210> 31 <211> 230 <212> PRT <213> Toxoplasma gondii Arg Arg Pro Tyr His Tyr Glu Met Leu Asp Ile Pro Ser Ile Arg Arg 5 10 1 15 Val Glu Leu Pro Gly Ala Gln Val Arg Met Pro Met Ala Lys Glu Leu 20 25 Val Arg Asp Trp Gly Ser Val Val Gln Gln Gln Thr Thr Ser Asp Ser 35 40 Ser Ser Asp Thr Pro Ala Thr Arg Ser Arg Ser Ala Glu Ala Leu Cys 55 Val Phe Ser Thr Pro Cys Thr Ala Asp Ser Asp Gln Arg Met Lys Gly 70 75 Arg His Tyr Pro Gln Ser Tyr His Thr Pro Arg Asp Ser Ala Thr Lys 85 95 Arg Glu Lys Pro Leu Lys Ser Thr Phe Ile Trp Gly Thr Thr Val Glu 100 105 110 Asp Arg Asn His Pro Ile Ser Pro Asp Pro Phe Ser Arg Leu Gln Gly 115 120 125 Cys Gly Gln Thr Leu Gln Asp Glu Leu Pro Ser Ala Arg Thr Arg Pro 135 140 130 Gly Trp Ala Ala Leu Asp Ser Arg Leu Lys Asn Lys Asp Pro Gln Ile

145 150 155 160

Ser Ala Gly Asp Glu Ala Ala Lys Val Asp Asp Thr Ser Ala Glu Pro
165 170 175

Cys Leu Gly Thr Val Pro Ser Phe Cys Arg Leu Val Thr Ser His Asp 180 185 190

Leu Leu Glu Ala Gly Ala Gln Val Arg Val Leu Gly Pro Thr Thr Asp 195 200 205

Pro Glu Thr Glu Thr Ala Ser Gln Leu Gln Thr Thr Glu Leu Ala Thr 210 . 215 220

Leu Thr Thr Val Asp Pro 225 230

<210> 32

<211> 313

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(162)

<400> 32

cgc agg aat aat cct gac ggt cag acg cag cgg ttc gtg cag aca gtg 48
Arg Arg Asn Asn Pro Asp Gly Gln Thr Gln Arg Phe Val Gln Thr Val
1 5 10 15

aag caa tgg cag agt gta aaa agc aga acc aga gcg tgt ctg tcg gcc 96 Lys Gln Trp Gln Ser Val Lys Ser Arg Thr Arg Ala Cys Leu Ser Ala 20 25 30

aaa gga aag aga agg caa atc aca cag cga ata aac ctc acc tct gtc 144 Lys Gly Lys Arg Arg Gln Ile Thr Gln Arg Ile Asn Leu Thr Ser Val 35 40 45

tcg cac ccc gaa gca acg taggagagcc actggtgccg ccactctgtg 192
Ser His Pro Glu Ala Thr
50

ctgacaaaaa agaaccggcc cttcttcggc aggggcgtag ccagtctgca gacatttcaa 252

tttcgaagcg accggaagca gtgaaatttc cagggaagac gcccaggaga cgtcaacagc 312

313

<210> 33

<211> 54

<212> PRT

<213> Toxoplasma gondii

<400> 33

Arg Arg Asn Asn Pro Asp Gly Gln Thr Gln Arg Phe Val Gln Thr Val

Lys Gln Trp Gln Ser Val Lys Ser Arg Thr Arg Ala Cys Leu Ser Ala 20 25 30

Lys Gly Lys Arg Arg Gln Ile Thr Gln Arg Ile Asn Leu Thr Ser Val 35 40 45

Ser His Pro Glu Ala Thr

<210> 34

<211> 389

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(195)

<400> 34

cgc tct cac gga ggc gca agt gag ttt tgg ctt tac ctc ttg aga aaa 48
Arg Ser His Gly Gly Ala Ser Glu Phe Trp Leu Tyr Leu Leu Arg Lys
1 5 10 15

cgg aac tct cca gaa gat cct cgt tcc gtc cgt cct cca cgt ccg tgt 96
Arg Asn Ser Pro Glu Asp Pro Arg Ser Val Arg Pro Pro Arg Pro Cys
20 25 30

gtc ttt cga gag atg gac aaa cag aga agc aga atc aag aaa gga ttc 144 Val Phe Arg Glu Met Asp Lys Gln Arg Ser Arg Ile Lys Lys Gly Phe 35 40 45

gca ttt gca ctt ggg tct gtc ttt tac ttc caa ggt cgt gaa ttt cat 192 Ala Phe Ala Leu Gly Ser Val Phe Tyr Phe Gln Gly Arg Glu Phe His 50 55 60

W	O 99/	/3263 3	3												PCT/U	JS98/27137
gcg Ala 65	tga	cgaa	taa	gaga	gaca	gg a	gtag	gccg	c aa	cttc	tcgt	ctc	ttgg	cag		245
ttt	tttccgattt ctcttccttc cgaagccctt gctgccaagc actccatccg gtccggttgg															305
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gcgtgtctgc atatctcttc cagg														389		
<210	<210> 35															
<211> 65																
<212> PRT																
<213> Toxoplasma gondii																
<400)> 35	5														
Arg 1	Ser	His	Gly	Gly 5	Ala	Ser	Glu	Phe	Trp 10	Leu	Tyr	Leu	Leu	Arg 15	Lys	
Arg	Asn	Ser	Pro 20	Glu	Asp	Pro	Arg	Ser 25	Val	Arg	Pro	Pro	Arg 30	Pro	Cys	
Val	Phe	Arg 35	Glu	Met	Asp	Lys	Gln 40	Arg	Ser	Arg	Ile	Lys 45	Lys	Gly	Phe	
Ala	Phe 50	Ala	Leu	Gly	Ser	Val 55	Phe	Tyr	Phe	Gln	Gly 60	Arg	Glu	Phe	His	
Ala 65																
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	> C[(546))												
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					Arg											48
ctc	tcc	сса	ctg	cct	ctc	gcc	gtc	cgc	gtc	gtt	cgc	ctc	cgg	ggg	agc	96

Leu Ser Pro Leu Pro Leu Ala Val Arg Val Val Arg Leu Arg Gly Ser																
Leu	Ser	Pro	Leu 20	Pro	Leu	Ala	Val	Arg 25	Val	Val	Arg	Leu	Arg 30	Gly	Ser	
		tgt Cys 35														144
		ctt Leu														192
		tct Ser														240
-		act Thr														288
		ggc Gly														336
		agc Ser 115														384
	_	gac Asp	_													432
	-	gtg Val														480
		tcc Ser			Ser					Ser						528
		ctg Leu	_	His	_	_										548

<210> 37

<211> 182

<212> PRT

<213> Toxoplasma gondii

<400> 37

Arg Ser Ser Ser Ers Arg Ser Leu Phe Phe Leu Ser Val Val Cys Val 1 5 10 15

Leu Ser Fra Lou Fro Leu Ala Val Arg Val Val Arg Leu Arg Gly Ser
25 30

Arg Gln Cy. :, ...u His Gly Gly Phe Ala Arg Arg Ala Ala Pro Arg ... 40 45

Ala Phe is a in : -i.y Arg Pro Thr Ser Leu Arg Ser Ser Gln Arg Thr 50 55 60

Pro Arg ::n Met Arg Arg Arg Ser Pro His Met Arg Cys Phe 65 70 75 80

Cys Glu T: · . . : Ser Ala Cys Cys Glu Arg Arg Lys Arg Ser Ala 90 95

Arg Asp .: 0.5 ... Gln Glu Ser Ala Lys Lys Ala Arg Pro Ser Asn 105 110

Pro Met ... : 2... lle His Ala Ser Val Asp Arg Val Gln Cys Gly 120 125

Gln Glr. A: -- +; Arg Ser Arg Arg Trp Pro Ala Ala Ser Thr Ser 130 135 140

Ala Gly Vi. ... ' a :le Arg Gly Arg Asn Ser Glu Val Pro Arg Val 145 150 155 160

Arg Ser ' Thr

<?10> 3-

<211> 3:

<212> f::::

<213> Tcs ndii

<220>

PCT/US98/27137

WO 99/32633 <220> <221> CDS <222> (1)..(285) <400> 38 cgg gat cca gct gca cct aac agc aca cag gct gtg gca gcc gct ygt 48 Arg Asp Pro Ala Ala Pro Asn Ser Thr Gln Ala Val Ala Ala Xaa 10 acc gtg gta gtg atg aaa acm gam gmw gaa gtg tcc ggt gac aac stc 96 Thr Val Val Met Lys Xaa Xaa Xaa Glu Val Ser Gly Asp Asn Xaa 20 25 agt caa ccg ggt agg sgt ccg ccg tcg cca aag ccg caw acg acg aag 144 Ser Gln Pro Gly Arg Xaa Pro Pro Ser Pro Lys Pro Xaa Thr Thr Lys ttt ccg cgg aga gag tca cca gac srg cag ggg acg agg cgg aga act 192 Phe Pro Arg Arg Glu Ser Pro Asp Xaa Gln Gly Thr Arg Arg Arg Thr 50 55 60 gaa agc cga ggc gct gtt agc agg gta tgg cca ggg gaa aac cag mga Glu Ser Arg Gly Ala Val Ser Arg Val Trp Pro Gly Glu Asn Gln Xaa 65 70 aga ctg tct gcc gtc gac gat tcg ata ccg gct aac cca tcg ctt 285 Arg Leu Ser Ala Val Asp Asp Ser Ile Pro Ala Asn Pro Ser Leu 85 90 95 tgaacgggtg gcgccctgcg atccg 310

<210> 39

<211> 95

<212> PRT

<213> Toxoplasma gondii

<400> 39

Arg Asp Pro Ala Ala Pro Asn Ser Thr Gln Ala Val Ala Ala Xaa 1

Thr Val Val Met Lys Xaa Xaa Glu Val Ser Gly Asp Asn Xaa 25 30

Ser Gln Pro Gly Arg Xaa Pro Pro Ser Pro Lys Pro Xaa Thr Thr Lys 35 40

Phe Pro Arg Arg Glu Ser Pro Asp Xaa Gln Gly Thr Arg Arg Arg Thr

50 55 60

Glu Ser Arg Gly Ala Val Ser Arg Val Trp Pro Gly Glu Asn Gln Xaa 65 70 75 80

Arg Leu Ser Ala Val Asp Asp Ser Ile Pro Ala Asn Pro Ser Leu 85 90 95

<210> 40

<211> 220

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(219)

<400> 40

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Arg Asp Pro Cys Leu Ser Val Arg Asp Ile Glu Arg Met Phe Arg Ile
1 5 10 15

tgt cac cat cgt tct ctg tct cgc ctc ctt ggc gcc tct gtt gct tgg 96 Cys His His Arg Ser Leu Ser Arg Leu Leu Gly Ala Ser Val Ala Trp 20 25 30

gat gca gtt gac tgc tct tcg gct tcg tcg cgc aca cac tgg tcc ttg 144 Asp Ala Val Asp Cys Ser Ser Ala Ser Ser Arg Thr His Trp Ser Leu 35 40 45

ctt gcg tct gag ctc cct tcc gaa cgg gtt ctt ttt cga ctg cag gtt 192 Leu Ala Ser Glu Leu Pro Ser Glu Arg Val Leu Phe Arg Leu Gln Val 50 55 60

ctt cta aaa ttg cca gtt ccc gat ccc g Leu Leu Lys Leu Pro Val Pro Asp Pro 65 70

<210> 41

<211> 73

<212> PRT

<213> Toxoplasma gondii

<400> 41

Arg Asp Pro Cys Leu Ser Val Arg Asp Ile Glu Arg Met Phe Arg Ile
1 5 10 15

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Cys His Hr: A:g Ser Leu Ser Arg Leu Leu Gly Ala Ser Val Ala Trp
            20
Asp Ala Lil Aug Cys Ser Ser Ala Ser Ser Arg Thr His Trp Ser Leu
                          40
Leu Ala ...: Leu Pro Ser Glu Arg Val Leu Phe Arg Leu Gln Val
     50
                                         60
Leu Leu Ly : : : !ro Val Pro Asp Pro
 65
                   70
<210> 4.
<211> 64.
<212> THA
<213> 7 . : . . 7 . jondii
<220:-
<223 to 200 at 19, 23, 27, 28, 29, 41, 86 and 88
<220>
<223: X..
        ... wn at 7, 8, 9, 10, 14, 29 and 30
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<221> ~t
<222> : : .
<400 - 4.
egg care and the standard action and the atg
Arg A:: . : Met Glu Xaa Xaa Xaa Xaa Thr Thr Ser Xaa Cys Met
                                 10
                                                   15
             ..t gca aac tat aga cac aaa caa ang naa aat acn
gta 7:
                                                           96
               : Ala Asn Tyr Arg His Lys Gln Xaa Xaa Asn Xaa
Val G.
                             25
                                               30
             nggggangtn ggggacagan aaatngteet teagttntea
                                                           152
Trp G.
tctt::
             nacgcaatac agcgggcgca gcggctcatc acaccantac 212
           :...ıca enthtettet etetteangt etethtacea ettetaceae 272
ctgcac ·
           .....ca caaaacacat ttgaacgatg tgaccaaaat gatccacaaa 332
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aacgtctaat ccagagtcct ctccgctcaa aaacacgatt gtttcgtcac atggaacctc 452
agcaaattca ggcgccagga cggcctccct tcaaacgtcn taatccagag tcntttccgn 512
tccatcccca cnttntgccc nttcacgttt ccagtggtgg catgtcatcg tctccccctg 572
tcaacgtccc atcacctgag tacaggcgcg aagcagcgga cagctgttct tccatctccc 632
tgtattccgg

<210> 43

<211> 34

<212> PRT

<213> Toxoplasma gondii

<400> 43

Arg Arg Glu Thr Met Glu Xaa Xaa Xaa Xaa Thr Thr Ser Xaa Cys Met

1 5 10 15

Val Gly Thr Gln Asn Ala Asn Tyr Arg His Lys Gln Xaa Xaa Asn Xaa 20 25 30

Trp Gly

<210> 44

<211> 381

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<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(81)

<400> 44

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Arg Ile His Lys Asn Thr Ile Val Ser Ser His Gly Thr Ser Ala Asn
1 5 10 15

tca ggc gcc agg acg gcc tcc ctt caa acg tcc taatccagag tcctctccgc 101 Ser Gly Ala Arg Thr Ala Ser Leu Gln Thr Ser 20 25

tecatececa cettetgece etteaegttt ceagtggtgg catgteateg tetececetg 161

<210> 45

<211> 27

<212> PRT

<213> Toxoplasma gondii

<400> 45

Arg Ile His Lys Asn Thr Ile Val Ser Ser His Gly Thr Ser Ala Asn
1 5 10 15

Ser Gly Ala Arg Thr Ala Ser Leu Gln Thr Ser

<210> 46

<211> 432

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(255)

<400> 46

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Phe Phe Ser Arg Cys Arg Arg Val Pro Glu Asn Val Lys Leu Leu Val
1 5 10 15

tac aag ttg att aac cct tct gtc gag gcc cgc ctg ctc gcc ttg caa 96
Tyr Lys Leu Ile Asn Pro Ser Val Glu Ala Arg Leu Leu Ala Leu Gln
20 25 30

gcg ata gag act ccg gaa tac agg gag atg gaa gaa cag ctg tcc gct 144 Ala Ile Glu Thr Pro Glu Tyr Arg Glu Met Glu Glu Gln Leu Ser Ala 35 40 45

gct tcg cgc ctg tac tca ggt gat ggg acg ttg aca ggg gga gac gat 192
Ala Ser Arg Leu Tyr Ser Gly Asp Gly Thr Leu Thr Gly Gly Asp Asp
50 55 60

gac atg cca cca ctg aaa cgt gaa ggg gca gaa ggt ggg gat gga gcg Asp Met Pro Pro Leu Lys Arg Glu Gly Ala Glu Gly Gly Asp Gly Ala 70 75 gag agg act ctg gat taggacgttt gaagggaggc cgtcctggcg cctgaatttg 295 Glu Arg Thr Leu Asp 85 ctgaggttcc atgtgacgaa acaatcgtgt ttttgagcgg agaggactct ggattaggac 355 gtttgaaggg aggccgtcct ggcgcctgaa tttgctgagg tttcatgtga cgaaacaatc 415 gtgtttttgt ggatccg 432 <210> 47 <211> 85 <212> PRT <213> Toxoplasma gondii <400> 47 Phe Phe Ser Arg Cys Arg Arg Val Pro Glu Asn Val Lys Leu Leu Val 10 Tyr Lys Leu Ile Asn Pro Ser Val Glu Ala Arg Leu Leu Ala Leu Gln 25 Ala Ile Glu Thr Pro Glu Tyr Arg Glu Met Glu Glu Gln Leu Ser Ala 40 Ala Ser Arg Leu Tyr Ser Gly Asp Gly Thr Leu Thr Gly Gly Asp Asp 50 55 Asp Met Pro Pro Leu Lys Arg Glu Gly Ala Glu Gly Gly Asp Gly Ala 65 70 75 Glu Arg Thr Leu Asp 85 <210> 48 <211> 282 <212> DNA <213> Toxoplasma gondii <220>

41

<221> CDS

<222> (1)..(105)

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Arg Arg Ala Ala Ser Gln Glu Arg Phe Ala Ala Ala Cys Gly Gln Gln
1 5 10 | 48 |
| agc ctt acc ctc gag ttt tct ctc gtg gct gcc gac gtc ggc gac gcc
Ser Leu Thr Leu Glu Phe Ser Leu Val Ala Ala Asp Val Gly Asp Ala
20 25 30 | 96 |
| gcg aac tcc tgagatcaaa cacacaaaaa ggccctcgtt gaaacatccc
Ala Asn Ser
35 | 145 |
| cacgcacgag cagaaggacg cgagcaagaa aacgtctcca gccttctctt gcggtcgctt | 205 |
| gcaageggga gtgtegtete eetetgtett tetetgtgta etegaageee agegaettee | 265 |
| ttgtcgagtt tctccgg | 282 |
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| <400> 49 | |
| Arg Arg Ala Ala Ser Gln Glu Arg Phe Ala Ala Ala Cys Gly Gln Gln 1 5 10 15 | |
| Ser Leu Thr Leu Glu Phe Ser Leu Val Ala Ala Asp Val Gly Asp Ala 20 25 30 | |
| Ala Asn Ser
35 | |
| <210> 50
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<212> DNA
<213> Toxoplasma gondii | |
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| <pre><220> <221> CDS <222> (1)(213)</pre> | |

| < 4 | 101 | 0> 5 | 0 | | | | | | | | | | | | | | |
|---------|-----|-------|-------|-----------|-------|-----------|--------------|--------|-----------|--------|--------|-------|-------|------------|-------|-------|-----|
| tt | t | ttt | tsg | agg | tgc | cgg | cgg | gtg | cca | gag | aam | gtg | aaa | ttc | tgg | ttt | 48 |
| Ph | | Phe | Xaa | Arg | Cys | Arg | Arg | Val | Pro | Glu | Xaa | Val | Lys | Phe | Trp | Phe | |
| | 1 | | | | 5 | | | | | 10 | | | | | 15 | | |
| | | | | | | | | | | | | | | gcc | | | 96 |
| \s | n | Lys | Leu | Ile
20 | Asn | Pro | Ser | Val | Glu
25 | Ala | Arg | Leu | Phe | Ala
30 | Leu | Gln | |
| ~~ | .~ | 2+2 | ~~~ | | 222 | ~~~ | * | | | | | | | | | | |
| | | | | | | | | | | | | | | ctg
Leu | | | 144 |
| | | | 35 | | | | , 7 - | 40 | | | | 014 | 45 | Dea | DCI | LIG | |
| gc | t | tcg | cgc | ctg | tac | tca | ggt | gat | ggg | acg | ttg | aca | ggg | gga | gac | gat | 192 |
| | | Ser | | | | | Gly | | | | | Thr | | Gly | | | |
| | | 50 | | | | | 55 | | | | | 60 | | | | | |
| | | | | | | | | tgaa | gggg | ca ç | gaago | gtggg | gg at | ggag | gegga | à | 243 |
| ۱s
6 | - | Met | Pro | Pro | Leu | Glu
70 | Thr | | | | | | | | | | |
| Ū | • | | | | | , 0 | | | | | | | | | | | |
| ga | gg | acto | etg g | gatta | aggac | g tt | tgaa | ıggga | ggc | cgto | ctg | gcgc | ctga | aat t | tgct | gaggt | 303 |
| tc | ca | tgtg | gac c | gaaac | caato | g to | gtttt | tgaç | g egg | agaç | gac | tctg | gatt | ag ç | gacgt | ttgaa | 363 |
| gg | ga | ggco | gt d | ctg | gegee | t ga | attt | gcto | g ago | ıttto | atg | tgac | gaaa | aca a | ıtcgt | gttt | 423 |
| tσ | t c | idato | oct o | יררת: | ataca | | at cac | raat = | | .at ac | na art | ata | | | | | 466 |
| - 9 | | , 9 | | Joge | | | 95 | ,9900 | . ege | acac | agt | gee | | | | | 466 |
| <2 | 10 |)> 51 | _ | | | | | | | | | | | | | | |
| <2 | 11 | .> 7] | L | | | | | | | | | | | | | | |
| | | ?> PF | | | | | | | | | | | | | | | |
| <2 | 13 | 3> Tc | goxo | lasma | a gor | ndii | | | | | | | | | | | |
| | |)> 51 | | | | | | | | | | | | | | | |
| | | Phe | Xaa | Arg | | Arg | Arg | Val | Pro | | Xaa | Val | Lys | Phe | - | Phe | |
| | 1 | | | | 5 | | | | | 10 | | | | | 15 | | |
| As | n | Lys | Leu | Ile | Asn | Pro | Ser | Val | Glu | Ala | Arg | Leu | Phe | Ala | Leu | Gln | |
| | | | | 20 | | | | | 25 | | | | | 30 | | | |
| Al | a | Ile | Glu | Xaa | Pro | Glu | Tyr | Arg | Glu | Met | Glu | Glu | Gln | Leu | Ser | Ala | |
| | | | 35 | | | | | 40 | | | | | 45 | | | | |
| Al | a | Ser | Arg | Leu | Tyr | Ser | Gly | Asp | Gly | Thr | Leu | Thr | Gly | Gly | Asp | Asp | |
| | | 50 | | | - | | 55 | = | | | | 60 | - | _ | - F. | £- | |

Asp Met Pro Pro Leu Glu Thr 65 70 <210> 52 <211> 539 <212> DNA <213> Toxoplasma gondii <220> <223> Xaa = unknown at 8, 9 and 16 <220> <221> CDS <222> (1)..(60) <400> 52 gat age aca egg aat gga tge ntg grg gtt ggg age gae tat att tnt Asp Ser Thr Arg Asn Gly Cys Xaa Xaa Val Gly Ser Asp Tyr Ile Xaa tat ttg gtg ctt taaagctcca actacaggac ctgaagagga atactccatc 100 Tyr Leu Val Leu 20 gaattettgt teteattgtg eeggegggea eeagagaaeg tgaaaetaet ggtttacaag 160 ttgattaacc cttctgtcga ggcccgcctg tcgccttgca agctacggag actccggaat 220 acagggagat ggaagaacag ctgtccgctg cttcgcgcct gtactcaggt gatgggacgt 280 tgacaggggg agacgatgac atgccaccac cggaaacgtg aaggggcaga aggtggggat 340 ggagcggaga ggactctgga ttaggacgtt tgaagggagg ccgtcctggc gcctgaattt 400 tgctgaggtt tcatgtgacg aaacaatcgt gtttttgtgg atccggaatt ccggatcggg 460 gaattteete teacaceget tggggeegag acaegegeag agaegttgtt gggeeteeae 520 aacacagggg ggattaagg 539

<210> 53 <211> 20 <212> PRT

<213> Toxoplasma gondii

<400> 53

Asp Ser Thr Arg Asn Gly Cys Xaa Xaa Val Gly Ser Asp Tyr Ile Xaa 1 5 10 15

Tyr Leu Val Leu 20

<210> 54

<211> 1233

<212> DNA

<213> Toxoplasma gondii

<400> 54

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ttcgagtgct ctttcgccgt tgcagtgct gacattgcgg cgtgggcaaa ggatagaagt 1080 gacgacctct gacatggcag tgaaggtggc agagactcgc ggaaaatcca aaaactctct 1140 gccgtttcgg tcgaggaatc acctttcttt ttttcgtctc tggacccgcc tccgtggtgt 1200 tcccttgccc ttgcaagccg ctgctatgta gcg 1233

<210> 55 <211> 411 <212> DNA <213> Toxoplasma gondii <220>

<221> CDS <222> (1)..(180)

<400> 55

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Arg Arg Pro Arg Leu Leu His Ile Gln Gly Met Ser Ser Cys Phe Gly
1 5 10 15

cct aag caa ccc gac ctt tat ctt ttg cac cag ctg tgc ttc ttt tac 96
Pro Lys Gln Pro Asp Leu Tyr Leu Leu His Gln Leu Cys Phe Phe Tyr
20 25 30

ttg tgt gaa tca ctg tgt aaa caa act gag aag cgt gta tgc atg gtc 144
Leu Cys Glu Ser Leu Cys Lys Gln Thr Glu Lys Arg Val Cys Met Val
35 40 45

gcc ttt gca tgt gga cga ggc cgc cgt cgc aca gcg tgattctcat 190
Ala Phe Ala Cys Gly Arg Gly Arg Arg Arg Thr Ala
50 55 60

ctctgttgcg tgggggcgcg gatgagaatc aactccttag tgtcacagca tcagtgcagt 250 gcgtggagca acaattcttt tcgtgcacag acaagacaca ccagatatga aagaacacta 310 acgggcactt accgttgtcc gtctatatat ttatatttag tcaatgctga gattagacct 370 agacttgtga gagagagtgt gaaacccaaa tgcctagatc c 411

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<223> Xaa = unknown at 51, 80 and 109

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<221> CDS

<222> (1)..(354)

<400> 57

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cac agc ggg aat gag aat cga ctc tgc gat aga agt ggg cgc cat gga 96 His Ser Gly Asn Glu Asn Arg Leu Cys Asp Arg Ser Gly Arg His Gly 20 25 30

atc aag gaa ccg agg cga agg agg ccc atg ctg ttg gcc gag gtg ccc 144

Ile Lys Glu Pro Arg Arg Arg Pro Met Leu Leu Ala Glu Val Pro

35 40 45

tgc ttg tkg gag ggc gcc cga cga aca ggg ttt cgt cag aga caa gca 192 Cys Leu Xaa Glu Gly Ala Arg Arg Thr Gly Phe Arg Gln Arg Gln Ala 50 55 60

ctt cgc tcg cgt ttg tgg ccc ctt gcc gtg cgg cac gcg tgc gta kcc 240 Leu Arg Ser Arg Leu Trp Pro Leu Ala Val Arg His Ala Cys Val Xaa 65 70 75 80

WO 99/32633 PCT/US98/27137 ttc aag aga gac tgc gga agc aga gag cca ttg agg ctg tcc gag 288 Phe Lys Arg Asp Cys Gly Ser Arg Glu Arg Pro Leu Arg Leu Ser Glu 85 90 gtc ggc tcc agc cga gct gga tcc gaa tcc tgc agc csg gga tcc act Val Gly Ser Ser Arg Ala Gly Ser Glu Ser Cys Ser Xaa Gly Ser Thr 100 105 110 agt cta gac gcg cac ccg tgacccactt caggaygcgg vmatwatrcm 384 Ser Leu Asp Ala His Pro 115 ggggcagatt tttwmggyta actatcattt ccccstwgtt gattmttcca gcaattg 441 <210> 58 <211> 118 <212> PRT <213> Toxoplasma gondii <400> 58 Arg Ile Glu Glu Ala Glu Ala Glu Thr Arg Ile Ala Glu Thr Gly Lys His Ser Gly Asn Glu Asn Arg Leu Cys Asp Arg Ser Gly Arg His Gly 25 Ile Lys Glu Pro Arg Arg Arg Pro Met Leu Leu Ala Glu Val Pro 40 Cys Leu Xaa Glu Gly Ala Arg Arg Thr Gly Phe Arg Gln Arg Gln Ala 50 Leu Arg Ser Arg Leu Trp Pro Leu Ala Val Arg His Ala Cys Val Xaa 65 70 75 Phe Lys Arg Asp Cys Gly Ser Arg Glu Arg Pro Leu Arg Leu Ser Glu 85 Val Gly Ser Ser Arg Ala Gly Ser Glu Ser Cys Ser Xaa Gly Ser Thr 105 110 Ser Leu Asp Ala His Pro 115

48

<210> 59 <211> 491

PCT/US98/27137 WO 99/32633

<212> DNA <213> Toxoplasma gondii <220> <221> CDS <222> (1)..(102) <400> 59 cgg cgg tat tat agg aca cgg ccg cct gct ggt aac atc tgt aat tta 48 Arg Arg Tyr Tyr Arg Thr Arg Pro Pro Ala Gly Asn Ile Cys Asn Leu 5 10 tca ttg tat ccc gtc gtc ccg tgt tcc aaa ctg gga atc ttt tct ttc 96 Ser Leu Tyr Pro Val Val Pro Cys Ser Lys Leu Gly Ile Phe Ser Phe 20 25 ctg agc tgacggtttg gcccgcaagc tcagccgagt acgaaaccat gattaggttg 152 Leu Ser gaggcctaat gtgctttttc gccagctgtc aaacgggcag ccaaggttga tttctctatg 212 agttgtcctc cgcgctctcg aattggtatt tcgtggtttc agattgaaag cgtcactcga 272 gctattacga ggcgtttcag caaaaaggaa gaatcactca gacacctgac cgacgcttga 332 tgtgctggcg gttgtgcaaa tccaggcatc actcaacgcc gatgctcagc aggacccatg 392 gatcttaaga ggttctgttc cactacatca gtgagagttt caaaaagaat cctgataact 452 acgcgcttct acaggtgccg cctttatggc aacgatccg 491 <210> 60 <211> 34 <212> PRT <213> Toxoplasma gondii <400> 60

Arg Arg Tyr Tyr Arg Thr Arg Pro Pro Ala Gly Asn Ile Cys Asn Leu 5 10 15

Ser Leu Tyr Pro Val Val Pro Cys Ser Lys Leu Gly Ile Phe Ser Phe 20 25

Leu Ser

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<211> 357
<212> DNA
<213> Text; labma gondii
<220>
<221> CD.
<222> (11.....
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egg atc 1- ": agt etc ttt ggg etc eet gee gea tge agg eat gaa
Arg Ile Al. Der Leu Phe Gly Leu Pro Ala Ala Cys Arg His Glu
                                    10
agt gtc · : gag aca gag aag gaa gtg cag agc gag cgt ggg
                                                                96
Ser Val . .: And Glu Thr Glu Lys Glu Val Gln Ser Glu Arg Gly
                                25
cga qui : ; : aaa ggc gca ggc gag aag gag acc ggc gta gac
                                                                144
Arg Glu / : .: Lys Gly Ala Gly Glu Lys Glu Thr Gly Val Asp
                            40
gga gt: , ' :: cag gtc tta gcg ctc act aag ggt gaa cct gaa
                                                                192
Gly Val Tr
               Gln Val Leu Ala Leu Thr Lys Gly Glu Pro Glu
    50
                        55
                                            60
gcg gcu :, ; ; aga gaa gag gac gag gga aag gga gaa gac aga
                                                                240
             Arg Glu Glu Asp Glu Gly Lys Gly Glu Asp Arg
65
                    70
                                        75
           · ::: gcg agg cga gag aaa gag gcg gct cga gtc atg
                                                                288
            . I'v Ala Arg Arg Glu Lys Glu Ala Ala Arg Val Met
                . .
                                    90
tcc a:
                 : tat gcc gaa gcc acc gac aca aca gct gca tgc
                                                                336
Ser :::
               : Tyr Ala Glu Ala Thr Asp Thr Thr Ala Ala Cys
                               105
aga 😋 i
               : ctc gcc tcg ggg gtc gaa gag aag aca cag gat
                                                                384
Arg A.:
                Leu Ala Ser Gly Val Glu Glu Lys Thr Gln Asp
                           120
ccg
                                                                387
Pro
<210> €.
<211> 1. ·
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<212> PRT

<213> Toxoplasma gondii

<400> 62

Arg Ile Ala Leu Ser Leu Phe Gly Leu Pro Ala Ala Cys Arg His Glu
1 5 10 15

Ser Val Ser Pro Arg Glu Thr Glu Lys Glu Val Gln Ser Glu Arg Gly
20 25 30

Arg Glu Arg Thr Gln Lys Gly Ala Gly Glu Lys Glu Thr Gly Val Asp 35 40 45

Gly Val Thr Gly Glu Gln Val Leu Ala Leu Thr Lys Gly Glu Pro Glu 50 55 60

Ala Ala Glu Glu Ala Arg Glu Glu Asp Glu Gly Lys Gly Glu Asp Arg 65 70 75 80

Trp Tyr Glu Glu Gly Ala Arg Arg Glu Lys Glu Ala Ala Arg Val Met
85 90 95

Ser Thr Pro Gln Thr Tyr Ala Glu Ala Thr Asp Thr Thr Ala Ala Cys 100 105 110

Arg Asp Glu Arg Glu Leu Ala Ser Gly Val Glu Glu Lys Thr Gln Asp 115 120 125

Pro

<210> 63

<211> 417

<212> DNA

<213> Toxoplasma gondii

<220>

 $\langle 223 \rangle$ N = unknown at 72, 74, 139 and 141

<220>

<223> At locations 25 and 47, Xaa = unknown

<220>

<221> CDS

<222> (1)..(417)

<223> Xaa = unknown at 25 and 47

<400> 63																
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	gaa Glu	-		-	-				_	_	-	-			_	96
-	agt Ser						-	-	_			-	-		-	144
-	cga Arg 50	-			-	_		_	_		-		-	-		192
-	cag Gln			•			-				-	-				240
	gag Glu	_						_		-		-		_	-	288
	gcg Ala															336
-	ggc Gly															384
_	acg Thr 130		_	-			_									417
<21: <21: <21:	0> 6. 1> 1: 2> P! 3> To	39 RT oxop	lasm	a goi	ndii											
	Ala		Ala	Val	Ala	Met	Glu	Glu	Ala		Ala	Pro	Gly	Gln 15	Pro	

Pro Glu Glu Gly Asp Asp Gly Xaa Xaa Gln Gln Arg Leu Glu Ile Ala

20 25 30

Leu Ser Leu Phe Gly Leu Pro Ala Ala Cys Arg His Glu Ser Xaa Ser 35 40 45

Pro Arg Glu Thr Glu Lys Glu Val Gln Ser Glu Arg Gly Arg Glu Arg 50 55 60

Thr Gln Lys Gly Ala Gly Glu Lys Glu Thr Gly Val Asp Gly Val Thr
65 70 75 80

Gly Glu Gln Leu Leu Ala Leu Thr Lys Gly Glu Pro Glu Ala Ala Glu 85 90 95

Glu Ala Arg Glu Glu Asp Glu Gly Lys Gly Glu Asp Arg Trp Asn Glu
100 105 110

Glu Gly Ala Arg Arg Glu Lys Glu Ala Ala Arg Val Met Ser Thr Pro 115 120 125

Gln Thr Tyr Ala Glu Ala Thr Asp Thr Thr Ala 130 135

<210> 65

<211> 416

<212> DNA

<213> Toxoplasma gondii

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<223> N = unknown at 74 and 107

<220>

<221> CDS

<222> (1)..(414)

<400> 65

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Pro Asp Arg Gly Arg Glu Glu Arg Glu Gly Glu Glu Glu Ser Ala Glu
1 5 15

gct ttg cca gac cat aag cgg ggg cca gga aaa gag ctg gag gaa ggc 96 Ala Leu Pro Asp His Lys Arg Gly Pro Gly Lys Glu Leu Glu Glu Gly 20 25 30

cga gac tcg cag gtc cgt ggt gag gag agc ggg cgc agc tcg ctt tcg 144 Arg Asp Ser Gln Val Arg Gly Glu Glu Ser Gly Arg Ser Ser Leu Ser 35 40 45

cag gag agg gaa agt ttt cgt tct cag cgn gtc t Gln Glu Arg Glu Ser Phe Arg Ser Gln Xaa Val S 50 55	cg gct gag ggt cag 192 er Ala Glu Gly Gln 60												
gag gtg gag gca gcn tct gtc aag gcg ctt gaa g Glu Val Glu Ala Xaa Ser Val Lys Ala Leu Glu G 65 70 75	ag gca aag tcg aac 240 lu Ala Lys Ser Asn 80												
gac aga ccc gac ggc gag agc aac gag ctg cgt co Asp Arg Pro Asp Gly Glu Ser Asn Glu Leu Arg An 85 90													
agc cag aca gag caa gaa ggc tcc gtc gag aaa ga Ser Gln Thr Glu Gln Glu Gly Ser Val Glu Lys Gl 100 105													
gcg acg atg aac gac caa gac gag aca ggg aag ga Ala Thr Met Asn Asp Gln Asp Glu Thr Gly Lys Gl 115 120	aa aaa caa gac caa 384 Lu Lys Gln Asp Gln 125												
cga gag gtg cct gtg ccc cgc gct ctt cgc tt Arg Glu Val Pro Val Pro Arg Ala Leu Arg 130 135	416												
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<400> 66 Pro Asp Arg Gly Arg Glu Glu Arg Glu Gly Glu Gl 1 5 10	u Glu Ser Ala Glu 15												
Pro Asp Arg Gly Arg Glu Glu Arg Glu Gly Glu Gl	15												
Pro Asp Arg Gly Arg Glu Glu Arg Glu Gly Glu Gl 1 5 10 Ala Leu Pro Asp His Lys Arg Gly Pro Gly Lys Gl	15 u Leu Glu Glu Gly 30												
Pro Asp Arg Gly Arg Glu Glu Arg Glu Gly Glu Gl 1 5 10 10 10 10 10 10 10 10 10 10 10 10 10	15 Lu Leu Glu Glu Gly 30 Eg Ser Ser Leu Ser 45												
Pro Asp Arg Gly Arg Glu Glu Arg Glu Gly Glu Gl 1 5 10 10 10 10 10 10 10 10 10 10 10 10 10	15 Lu Leu Glu Glu Gly 30 Eg Ser Ser Leu Ser 45 er Ala Glu Gly Gln												

90 95

Ser Gln Thr Glu Gln Glu Gly Ser Val Glu Lys Glu Gly Thr Ser Glu
100 105 110

Ala Thr Met Asn Asp Gln Asp Glu Thr Gly Lys Glu Lys Gln Asp Gln
115 120 125

Arg Glu Val Pro Val Pro Arg Ala Leu Arg 130 135

<210> 67

<211> 500

<212> DNA

<213> Toxoplasma gondii

<400> 67

ccgagaatca tgttacgcca tgtagacagc gtttagggag tgcagacatt ttaatctgga 60 cggagtccaa gtggacgcgg atgtagatat ctgtcgcagc acctccgcag ttgcgctagg 120 gattctgatg ctgctagttt taacatccaa aactctgact tcgcttggtg atctccaggt 180 gcatatacat gcgaaggcaa tcgtgtttgt gagaggcgaa tgtacgaatt tcagtgtctt 240 tgtgtggaag tcaagttccc ctgaaccagc tgcttgttt attctaccgc taatgtatga 300 agcttagcct cgtgtcctct tcgcccgtac acgagacacg atccaagagt catacaaatt 360 cttgcggcgg tgaggtaatt gtcaacagaa acaaaagtcg cgggtatctg tggtgtctct 420 gcttctgcac ttccaaggac cgccgcaagt tcggcccgat cggctggaac attcagtacg 480 agttcacgac ggaggatccg

<210> 68

<211> 321

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(219)

<400> 68

cgg cgg gac ttg cgg act tcg gtc tgg gac gct cgg gtg tac gta cac 48

• • •																
Arg 1	Arg	Asp	Leu	Arg 5	Thr	Ser	Val	Trp	Asp 10	Ala	Arg	Val	Tyr	Val 15	His	
_	gcg Ala															96
-	agg Arg															144
	cta Leu 50	_														192
-	aca Thr		_						tgaa	iggaa	at o	cacaç	gacat	c		239
acca	acct	tc c	eegee	gtgg	gc ta	aagg	jacco	tco	etgtç	gtat	gtac	agtt	tt t	ccaç	ggcgaa	299
agco	cgaga	aga c	cage	gaaac	ec go	J										321
<210> 69 <211> 73 <212> PRT <213> Toxoplasma gondii																
- 40)	,														
	0> 69 Arg		Leu	Arg 5	Thr	Ser	Val	Trp	Asp 10	Ala	Arg	Val	Tyr	Val 15	His	
Leu	Ala	Gly	Gly 20	Gln	Arg	Arg	Cys	Asn 25	Glu	Ser	Arg	Gly	Met 30	Glu	Glu	
Ala	Arg	Lys 35		Arg	Cys	Leu	Ala 40	Met	Arg	Cys	Gln	Trp 45	Thr	Ser	Ser	
Ala	Leu 50	Asp	Trp	Arg	Glu	Ser 55	Trp	Lys	Asn	Ala	Glu 60	Thr	Ala	Ser	His	
Val 65	Thr	Phe	Pro	Thr	Lys 70	Arg	Pro	Pro								
<21	0> 7	0														
<21	1> 5	13														

<212> DNA <213> Toxoplasma gondii <220> <221> CDS <222> (1)..(513) <400> 70 cgg gat cag gct tct atg cca ctg ccc ccg gcc ccc gaa gac ttt gac 48 Arg Asp Gln Ala Ser Met Pro Leu Pro Pro Ala Pro Glu Asp Phe Asp ctg cct cct atg cca ctg ccc gaa gca ccc gaa gac ttt gac cag gct 96 Leu Pro Pro Met Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala 20 25 30 cct atg cca ctg ccc gag gca ccc gaa gac ttt gac cag gct cct atg 144 Pro Met Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala Pro Met 35 40 cca ctg ccc gag gca ccc gaa gac ttt gac cag cct cct atg cca ctg Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Pro Pro Met Pro Leu 55 ccc gaa gca ccc gaa gac ttt gac cag gct cct atg cca ctg ccc gaa Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala Pro Met Pro Leu Pro Glu 65 70 gca ccc gaa gtc ttt gac cag gct cct atg cca ctg ccc gag gca ccc 288 Ala Pro Glu Val Phe Asp Gln Ala Pro Met Pro Leu Pro Glu Ala Pro 90 gaa gtc ttt gac cag gct cct atg cca ctg ccc gaa gca ccc gaa gac 336 Glu Val Phe Asp Gln Ala Pro Met Pro Leu Pro Glu Ala Pro Glu Asp 100 105 110 ttt gac cag gct cct atg cca ctg ccc gaa gca ccc gaa gtc ttt gac 384 Phe Asp Gln Ala Pro Met Pro Leu Pro Glu Ala Pro Glu Val Phe Asp 115 120 cag gct cct atg cca ctg ccc gag gca ccc gaa gac ttt gac cag gct 432 Gln Ala Pro Met Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala . 130 135 140

150

cct atg cca gtg ccc gag gca ccc gaa gac ttt gac cag gct cct gag

Pro Met Pro Val Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala Pro Glu

155

480

160

145

cca ctg ccc gag gca gcc gaa gaa ttt gat ccc Pro Leu Pro Glu Ala Ala Glu Glu Phe Asp Pro 165 170

513

<210> 71

<211> 171

<212> PRT

<213> Toxoplasma gondii

<400> 71

Arg Asp Gln Ala Ser Met Pro Leu Pro Pro Ala Pro Glu Asp Phe Asp

Leu Pro Pro Met Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala 20 25 30

Pro Met Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala Pro Met 35 40 45

Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Pro Pro Met Pro Leu 50 55 60

Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala Pro Met Pro Leu Pro Glu 65 70 75 80

Ala Pro Glu Val Phe Asp Gln Ala Pro Met Pro Leu Pro Glu Ala Pro 85 90 95

Glu Val Phe Asp Gln Ala Pro Met Pro Leu Pro Glu Ala Pro Glu Asp 100 105 110

Phe Asp Gln Ala Pro Met Pro Leu Pro Glu Ala Pro Glu Val Phe Asp 115 120 125

Gln Ala Pro Met Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala 130 135 140

Pro Met Pro Val Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala Pro Glu 145 150 155 160

Pro Leu Pro Glu Ala Ala Glu Glu Phe Asp Pro 165 170

<210> 72

<211> 528

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(528)

<400> 72

cga tct gaa cgt tgt gca acc gtt ggg gac cca ggt aca ggc gtc tcc 48
Arg Ser Glu Arg Cys Ala Thr Val Gly Asp Pro Gly Thr Gly Val Ser
1 5 10 15

aac act gag gcg ggg aag cgc cca cac tgg cgt ctc agg cac ctt 96
Asn Thr Glu Ala Gly Gly Lys Arg Pro His Trp Arg Leu Arg His Leu
20 25 30

caa tgc cac agg tat ccg gca tcc ttg gag aca gag ctt gag acg gag 144 Gln Cys His Arg Tyr Pro Ala Ser Leu Glu Thr Glu Leu Glu Thr Glu 35 40 45

aca ctc gca cac aca ccc aga gag ctt gtg gtg aca aat cga agc ttg 192
Thr Leu Ala His Thr Pro Arg Glu Leu Val Val Thr Asn Arg Ser Leu
50 55 60

ggg ttt gtc tcg ctt ctt cgc cag tcg ttc gcg tcg cag tca gaa gca 240 Gly Phe Val Ser Leu Leu Arg Gln Ser Phe Ala Ser Gln Ser Glu Ala 65 70 75 80

gtc aag gcg acc gcg gag acg ccg aca gag aca gag aca gtc ctt gtg $$ 288 Val Lys Ala Thr Ala Glu Thr Pro Thr Glu Thr Glu Thr Val Leu Val $$ 85 $$ 90 $$ 95

gcg ggc gag cgc aac acc gcg aaa gaa aga gag aga aaa ggg cag gac 336 Ala Gly Glu Arg Asn Thr Ala Lys Glu Arg Glu Arg Lys Gly Gln Asp 100 105 110

gaa gag gtt tcg cag aga gca gcg gag aac aag aga gga cga gtg gag 384 Glu Glu Val Ser Gln Arg Ala Ala Glu Asn Lys Arg Gly Arg Val Glu 115 120 125

gac aca gac tac cgg gag acg gat aag aaa gcc gag aaa gat gag cga 432 Asp Thr Asp Tyr Arg Glu Thr Asp Lys Lys Ala Glu Lys Asp Glu Arg 130 135 140

gaa gag aac ccc cga gga gac aca ggg gag cag aga agc gag aag cac 480 Glu Glu Asn Pro Arg Gly Asp Thr Gly Glu Gln Arg Ser Glu Lys His 145 150 155 160

acg aga gat tta ttg gga cag gag aga gag aac gca tgg gag atc ccg 528

Thr Arg Asp Leu Gly Gln Glu Arg Glu Asn Ala Trp Glu Ile Pro 165 170 175

<210> 73

<211> 176

<212> PRT

<213> Toxoplasma gondii

<400> 73

Arg Ser Glu Arg Cys Ala Thr Val Gly Asp Pro Gly Thr Gly Val Ser 1 5 10 15

Asn Thr Glu Ala Gly Gly Lys Arg Pro His Trp Arg Leu Arg His Leu 20 25 30

Gln Cys His Arg Tyr Pro Ala Ser Leu Glu Thr Glu Leu Glu Thr Glu 35 40 45

Thr Leu Ala His Thr Pro Arg Glu Leu Val Val Thr Asn Arg Ser Leu 50 55 60

Gly Phe Val Ser Leu Leu Arg Gln Ser Phe Ala Ser Gln Ser Glu Ala 65 70 75 80

Val Lys Ala Thr Ala Glu Thr Pro Thr Glu Thr Glu Thr Val Leu Val
85 90 95

Ala Gly Glu Arg Asn Thr Ala Lys Glu Arg Glu Arg Lys Gly Gln Asp 100 105 110

Glu Glu Val Ser Gln Arg Ala Ala Glu Asn Lys Arg Gly Arg Val Glu 115 120 125

Asp Thr Asp Tyr Arg Glu Thr Asp Lys Lys Ala Glu Lys Asp Glu Arg 130 135 140

Glu Glu Asn Pro Arg Gly Asp Thr Gly Glu Gln Arg Ser Glu Lys His 145 150 155 160

Thr Arg Asp Leu Gly Gln Glu Arg Glu Asn Ala Trp Glu Ile Pro 165 170 175

<210> 74

<211> 375

<212> DNA

<213> Toxoplasma gondii

<220> <221> CDS <222> (1)3	.=c.				
<400> 74 ccg gag dan ' Pro Glu Glu T					
gat gtc gat : Asp Val Ala V			Cys Tyr Arg		
aag aag oot : Lys Lys A.a î					
aag gtt c . : Lys Val :: . 50					
aag gag : ··· Lys Glu A: - 7 65					
gac gaa a:				tgc aag ctg Cys Lys Leu 95	
cag cto Gln Leu L .					
aac gtc •. Asn Va! :.			gag cag acg Glu Gln Thr		375
<210> 75 <211> 17 <212> FFT <213> 7.5	. : bdi				
<400> 7: Pro Glu	. Dys	Ser Lys Thr	Thr Tyr Glu 10	Asp Ser Cys	Thr

Asp Val Ala Val Gln Val Pro Asp Thr Cys Tyr Arg Thr Val Asp Gln
20 25 30

Lys Lys Ala Tyr Lys Cys Lys Lys Thr Leu Thr Lys Asn Gln Cys Thr 35 40 45

Lys Val Pro Val Gln Val Pro Ser Thr Cys Thr Lys Thr Ala Met Ser 50 55 60

Lys Glu Ala Tyr Asp Cys Ser Lys Thr Glu Phe Arg Thr Glu Cys Thr 65 70 75 80

Asp Glu Val Glu Gln Val Pro Cys Met Gly Lys Glu Cys Lys Leu Arg 85 90 95

Gln Leu Lys Lys Lys Arg Val Cys Arg Gln Val Pro Phe Thr Ser Lys 100 105 110

Asn Val Cys Tyr Lys Asn Val Pro Thr Glu Gln Thr Ser 115 120 125

<210> 76

<211> 543

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(267)

<400> 76

cga tcc aac agt tta cga ggt aca agg caa cag ccg aac ctc tac gag 48
Arg Ser Asn Ser Leu Arg Gly Thr Arg Gln Gln Pro Asn Leu Tyr Glu
1 5 10 15

cac gtg tcc cca cgg ttc acg ctc tcc cat gga aaa gca aag cga ttc 96 His Val Ser Pro Arg Phe Thr Leu Ser His Gly Lys Ala Lys Arg Phe 20 25 30

ctc cat tat cac cac tgc cac tgc cat tcc agc cta aga atc cta cac 144
Leu His Tyr His His Cys His Cys His Ser Ser Leu Arg Ile Leu His
35 40 45

ttc aaa gac gaa ctt ttg cat cgt ccg tgc gtc tcc cgt ggc caa cac 192 Phe Lys Asp Glu Leu Leu His Arg Pro Cys Val Ser Arg Gly Gln His 50 55 60

cct caa gcc aaa aga gag ggc acc ttc tac act gcc cac gca atc acc 240
Pro Gln Ala Lys Arg Glu Gly Thr Phe Tyr Thr Ala His Ala Ile Thr
65 70 75 80

ctg tgc ggc ggc aca caa aag cga aac tgacacacgc tactgccgtt 287 Leu Cys Gly Gly Thr Gln Lys Arg Asn

ccggaaagtg gtctgaaaga aactgacaac agccgcaaag agacatttac ccggtgcctg 347
gcgtggtcaa aaatccggca taatggtttc tgcgcatcct ccattcagcc gcccaacatc 407
tgcggtcgtt cttccgtcga aactatgaca caacgagcct tgtggaacaa aacggttcgt 467
actgacgaca ttgcctgggt cggattcact gcatgtttgc cagggtgcat ttccacggtg 527
ctctgcgtcg atcccg

<210> 77

<211> 89

<212> PRT

<213> Toxoplasma gondii

<400> 77

Arg Ser Asn Ser Leu Arg Gly Thr Arg Gln Gln Pro Asn Leu Tyr Glu
1 5 10 15

His Val Ser Pro Arg Phe Thr Leu Ser His Gly Lys Ala Lys Arg Phe
20 25 30

Leu His Tyr His His Cys His Cys His Ser Ser Leu Arg Ile Leu His
35 40 45

Phe Lys Asp Glu Leu Leu His Arg Pro Cys Val Ser Arg Gly Gln His 50 55 60

Pro Gln Ala Lys Arg Glu Gly Thr Phe Tyr Thr Ala His Ala Ile Thr 65 70 75 80

Leu Cys Gly Gly Thr Gln Lys Arg Asn 85

<210> 78

<211> 573

<212> DNA

<213> Toxoplasma gondii

<220>														
<221> CDG														
<222> (1, ^[3])														
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ccg gcg tim tim agc	tcg agg ctg	ggc aag ctg g	ct tac gac gat gca 48											
Pro Ala Der Der	Ser Arg Leu		· · · · · · · · · · · · · · · · · · ·											
1 5		10	15											
gga ggt mil in maa	gcg agc tcg	cca cca tct t	ct aag ttg ttt gtt 96											
Gly Gly Gly Ar : Gly	Ala Ser Ser	Pro Pro Ser S	er Lys Leu Phe Val											
. 1		25	30											
toc cca :	agg tca cgg	atg gca gat c	aa cga aaa cct gca 144											
Ser Pro Va. Va Aup	Arg Ser Arg	Met Ala Asp G	ln Arg Lys Pro Ala											
	40		45											
coo gaa kaa ta ta ta ta	aat cac gat	tcg gaa tgc t	gt tgc cta cgc tgt 192											
Pro Glu . · · · · · · · · · · · · · · · · · ·	Asn His Asp	Ser Glu Cys C	ys Cys Leu Arg Cys											
59	55		60											
ctg agt is: **; am;	ctg atg atg	gca cag ctc t	gc agg cct gca cct 240											
Leu Ser 7 r	Leu Met Met	Ala Gln Leu C	ys Arg Pro Ala Pro											
65	70	75	80											
gta acc '''' ra	aca gag agg	aac cta ttt q	ga gat aat ggc aga 288											
Val Thr Land Val		-												
ć ~		90	95											
gac qtc : ' . ':;	gag ggt tca	tac aga ttt t	tt tct gga aat gca 336											
			The Ser Gly Asn Ala											
•	-	105	110											
tog ac*	ota caa tto	tcc cct cac c	gt gtc atc gat gcc 384											
			arg Val Ile Asp Ala											
	120		125											
007.03	nat ata aaa	ant tac aga c	gca gcc cct gaa gac 432											
			Ala Ala Pro Glu Asp											
13	135		140											
			ngt ngg nng nto ngg 490											
ggg aca			agt agc aac ata aca - 480 Ser Ser Asn Ile Thr											
145	150	155	160											
aaa acq : :::														
Lys Thr 1	Asn Gly Aro	g Asp Val Cys (siu Gly Leu Arg Lys											

165 170 175

ccg ttg cag gac gat tct gaa gga gtc caa caa cct ctt ccg ccg 573
Pro Leu Gln Asp Asp Ser Glu Gly Val Gln Gln Pro Leu Pro Pro
180 185 190

<210> 79

<211> 191

<212> PRT

<213> Toxoplasma gondii

<400> 79

Pro Ala Ser Ser Ser Ser Arg Leu Gly Lys Leu Ala Tyr Asp Asp Ala 1 5 10 15

Gly Gly Gly Arg Gly Ala Ser Ser Pro Pro Ser Ser Lys Leu Phe Val 20 25 30

Ser Pro Val Asn Asp Arg Ser Arg Met Ala Asp Gln Arg Lys Pro Ala 35 40 45

Pro Glu Gln Ser Ser Asn His Asp Ser Glu Cys Cys Cys Leu Arg Cys 50 55 60

Leu Ser Glu Lys Thr Leu Met Met Ala Gln Leu Cys Arg Pro Ala Pro 65 70 75 80

Val Thr Leu Ser Val Thr Glu Arg Asn Leu Phe Gly Asp Asn Gly Arg 85 90 95

Asp Val Val Glu Trp Glu Gly Ser Cys Gly Phe Phe Ser Gly Asn Ala 100 105 110

Ser Thr Arg Pro Ser Leu Gln Phe Ser Pro His Arg Val Ile Asp Ala 115 120 125

Pro Thr Ala Asn Asp Asp Met Arg Asp Cys Arg Ala Ala Pro Glu Asp 130 135 140

Gly Thr Gly Thr Ser Lys Ala Asn Ile His Arg Ser Ser Asn Ile Thr 145 150 155 160

Lys Thr Lys Glu Glu Asn Gly Arg Asp Val Cys Glu Gly Leu Arg Lys
165 170 175

Pro Leu Gln Asp Asp Ser Glu Gly Val Gln Gln Pro Leu Pro Pro 180 185 190

	<210> 80 <211> 1835															
	<212> DNA <213> Toxoplasma gondii															
<213	<213> Toxoplasma gondii															
<220	<220> <221> CDS															
<221																
<222	<222> (1) (1833)															
< 400)> 80)														
cgg	atc	agt	ggg	gac	cag	tac	tct	tgt	ctt	caa	cga	gga	gcg	gga	gga	48
Arg	Ile	Ser	Gly	Asp	Gln	Tyr	Ser	Cys	Leu	Gln	Arg	Gly	Ala	Gly	Gly	
1				5					10					15		
gac	aag	gag	aca	gca	acc	gag	aga	gaa	gag	agg	aac	aga	gaa	gat	gcg	96
Asp	Lys	Glu	Thr	Ala	Thr	Glu	Arg	Glu	Glu	Arg	Asn	Arg	Glu	Asp	Ala	
			20					25					30			
ccc	tcc	ttt	ctt	gaa	gga	gga	ctc	gga	gat	gac	gag	aca	gag	aga	gcg	144
Pro	Ser	Phe	Leu	Glu	Gly	Gly	Leu	Gly	Asp	Asp	Glu	Thr	Glu	Arg	Ala	
		35					40					45				
aag	caa	gcg	agt	gag	ttg	ccc	gcg	tct	ctt	tgc	tct	ttc	gcc	gca	gca	192
Lys	Gln	Ala	Ser	Glu	Leu	Pro	Ala	Ser	Leu	Cys	Ser	Phe	Ala	Ala	Ala	
	50					55					60					
cgc	agg	ggc	gcg	agc	cgc	gca	gag	aag	aca	ggc	gca	aag	ggg	gag	gaa	240
Arg	Arg	Gly	Ala	Ser	Arg	Ala	Glu	Lys	Thr	Gly	Ala	Lys	Gly	Glu	Glu	
65					70					75					80	
									-	_	_		cta		_	288
Ala	Arg	Glu	Lys		Val	Ser	Phe	Gly		Asp	Ser	Gly	Leu	Ser	Arg	
				85					90					95		
													gga			336
Gln	Val	Asp	Met	Asp	Ser	Ser	Gln	Glu	Ser	Val	Asn	Glu	Gly	Glu	Pro	
			100					105					110			
		-	_	_	-			_	_	-			gga	-		384
Leu	His		Arg	Ala	Ala	Gly		Asp	Ala	Glu	Gly	_	Gly	Ala	Glu	
		115					120					125				
													cga	-		432
Ala		Asp	Gly	Asp	Arg		Gly	Asp	Glu	Lys		Thr	Arg	Asp	Val	
	130					135					140					

W	O 99	/32633	3						PCT/	US98/27137
		gaa Glu								480
		gaa Glu								528
		tcg Ser								576
		gat Asp 195								624
		cta Leu								672
		gct Ala								720
		tct Ser								768
		gca Ala							-	816
		ggt Gly 275								864
		gaa Glu								912
		cag Gln								960
		aga Arg								1008

W	O 99/	32633	,												101/	00/0/2/
gag Glu																1056
aga Arg																1104
cgc Arg			gtg Val													1152
			cgc Arg													1200
gaa Glu	gaa Glu	gaa Glu	gag Glu	aga Arg 405	gaa Glu	cgc Arg	agg Arg	aga Arg	gtg Val 410	gag Glu	gaa Glu	gag Glu	aag Lys	gcg Ala 415	aga Arg	1248
			gaa Glu 420													1296
			cag Gln													1344
gag Glu	gaa Glu 450	Glu	aag Lys	gcg Ala	aga Arg	cag Gln 455	aga Arg	gag Glu	gaa Glu	gaa Glu	gag Glu 460	aga Arg	gaa Glu	cgc Arg	agg Arg	1392
	Val	Glu	gaa Glu	Glu	Lys	Glu	Arg	Glu	Arg	Gln	Glu					1440
			gta Val		Glu					Glu					Glu	1488
gag Glu	aga Arc	a gaa g Glu	cgc Arç 500	, Arg	aga Arg	gtg Val	gag Glu	gaa Glu 505	Glu	aag Lys	gag Glu	aga Arg	gag Glu 510	Arg	cag Gln	1536
			ı Lys					g Arq					Lys		g aga n Arg	1584

WO 99/32633 PCT/US98/27137 1632 Gln Arg Gln Glu Glu Glu Gly Arg Glu Arg Gln Arg Gly Glu Glu Arg 530 535 gaa gag aga gaa ttt caa cag cgc gag cgg gag ctg aag aca 1680 Glu Glu Arg Glu Arg Glu Phe Gln Gln Arg Glu Arg Glu Leu Lys Thr 545 550 555 cgg cta gta gag ctt cag aga gag cac gca gag tct gtt gaa acg tgg 1728 Arg Leu Val Glu Leu Gln Arg Glu His Ala Glu Ser Val Glu Thr Trp 565 atg aag gag caa gga gaa cga gaa agg cac ttg act cag gat tgg gag 1776 Met Lys Glu Gln Gly Glu Arg Glu Arg His Leu Thr Gln Asp Trp Glu 580 585 590 agg aaa ttg cat gcg ttt gaa gag cag agt cgg act gtg ttg ctc caa 1824 Arg Lys Leu His Ala Phe Glu Glu Gln Ser Arg Thr Val Leu Leu Gln 595 600 605 gag aga tcc cg 1835 Glu Arg Ser 610 <210> 81 <211> 611 <212> PRT <213> Toxoplasma gondii <400> 81 Arg Ile Ser Gly Asp Gln Tyr Ser Cys Leu Gln Arg Gly Ala Gly Gly 10 Asp Lys Glu Thr Ala Thr Glu Arg Glu Glu Arg Asn Arg Glu Asp Ala Pro Ser Phe Leu Glu Gly Gly Leu Gly Asp Asp Glu Thr Glu Arg Ala 35 40 Lys Gln Ala Ser Glu Leu Pro Ala Ser Leu Cys Ser Phe Ala Ala Ala 50 55

70

85

Arg Arg Gly Ala Ser Arg Ala Glu Lys Thr Gly Ala Lys Gly Glu Glu

Ala Arg Glu Lys Glu Val Ser Phe Gly Glu Asp Ser Gly Leu Ser Arg

75

90

65

Gln Val Asp Met Asp Ser Ser Gln Glu Ser Val Asn Glu Gly Glu Pro
100 105 110

Leu His Asp Arg Ala Ala Gly Glu Asp Ala Glu Gly Gly Gly Ala Glu
115 120 125

Ala Asn Asp Gly Asp Arg Glu Gly Asp Glu Lys Glu Thr Arg Asp Val 130 135 140

Glu Asp Glu Gly Glu Thr Arg Arg Ser Ser Ser Phe Ala Glu Gln Thr 145 150 155 160

Gly Asn Glu Arg Thr Glu Met Arg Thr Arg His Gly Gly Asp Glu Gly 165 170 175

Trp Thr Ser Lys Ser Asn Arg Phe Ala Phe Ala Cys Pro Arg Phe Ser 180 185 190

Lys Ser Asp Val Cys Cys Ser Pro Gln Ala Arg Leu Ser Leu Pro Glu 195 200 205

Gln Ser Leu Gly Ser Ser Pro Ser Ser Pro Ile Ser Val Thr Asn Asp 210 215 220

Val Tyr Ala Leu Phe Asp Ser Ser Ala Ser Pro Leu His Ala Gly Glu 225 230 235 240

Leu Ser Ser Leu Pro Gly Ala Val Ser Ala Ser Glu Arg Leu Leu Thr 245 250 255

Ala Pro Ala Glu Ile Gly Pro Ser Ala Ser Ser Ala Cys Leu Ser Val 260 265 270

Ser Cys Gly Pro Gly Glu Met Ser Pro Thr Ala Asp Thr Thr Arg His 275 280 285

Asp Ala Glu Glu Arg Glu Arg Arg Arg Ala Glu Glu Lys Glu Arg 290 295 300

Glu Arg Gln Glu Glu Glu Glu Arg Glu Arg Arg Arg Val Glu Glu 305 310 315 320

Lys Glu Arg Glu Arg Gln Glu Glu Glu Glu Arg Glu Arg Arg Arg Val 325 330 335

Glu Glu Glu Lys Ala Arg Gln Arg Glu Glu Asp Glu Arg Glu Arg Arg 340 345 350

Arg Val Glu Glu Glu Lys Ala Arg Gln Arg Glu Glu Glu Glu Arg Glu Arg Arg Val Glu Glu Lys Ala Arg Gln Arg Glu Glu Glu Glu Glu Arg Glu Arg Arg Arg Val Glu Glu Lys Ala Arg Gln Arg Glu Glu Glu Glu Arg Glu Arg Arg Arg Val Glu Glu Lys Ala Arg Gln Arg Glu Glu Glu Glu Glu Glu Gly Arg Arg Val Glu Glu Glu Lys Ala Arg Gln Arg Glu Glu Glu Glu Glu Arg Glu Gly Arg Arg Val Glu Glu Glu Lys Ala Arg Gln Arg Glu Glu Glu Glu Arg Glu Arg Arg Arg Val Glu Glu Glu Lys Glu Arg Glu Arg Gln Glu Glu Glu Arg Glu Arg Arg Arg Val Glu Glu Glu Lys Glu Arg Glu Arg Gln Glu Glu Glu Arg Glu Arg Arg Val Glu Glu Glu Lys Glu Arg Glu Arg Gln Glu Glu Glu Lys Arg Glu Arg Arg Val Glu Glu Lys Ala Arg Gln Arg Gln Glu Glu Gly Arg Glu Arg Gln Arg Gly Glu Glu Arg Glu Glu Arg Glu Arg Glu Phe Gln Gln Arg Glu Arg Glu Leu Lys Thr Arg Leu Val Glu Leu Gln Arg Glu His Ala Glu Ser Val Glu Thr Trp Met Lys Glu Gln Gly Glu Arg Glu Arg His Leu Thr Gln Asp Trp Glu Arg Lys Leu His Ala Phe Glu Glu Gln Ser Arg Thr Val Leu Leu Gln

Glu Arg Ser 610

<210> 82

<211> 604

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(336)

<400> 82

ccg atg caa ttt gtc tct cct tcc cct ttt gtg caa tcc gac tcc ccc 48 Pro Met Gln Phe Val Ser Pro Ser Pro Phe Val Gln Ser Asp Ser Pro 1 5 10 15

tct tcg ccc ttc gca caa tcg gct tca cct cct tcc gag tac caa 96 Ser Ser Pro Phe Ala Gln Ser Ala Ser Pro Pro Pro Ser Glu Tyr Gln 20 25 30

gac tot out too out cot ttg goa gaa too gto tog tog out cot ttg 144
Asp Ser Leu Ser Leu Pro Leu Ala Glu Ser Val Ser Ser Leu Pro Leu
35 40 45

gcg aaa cag gct tct cct ctt cac ttg aca caa cac cct tct ccc ctt 192
Ala Lys Gln Ala Ser Pro Leu His Leu Thr Gln His Pro Ser Pro Leu
50 55 60

cta tgg aca cag cgg gcc tct cca tct cct ttc ttg gtt caa cgg gat 240 Leu Trp Thr Gln Arg Ala Ser Pro Ser Pro Phe Leu Val Gln Arg Asp 65 70 75 80

tcg tca cct cct tct gcg tca atg cgg ctt tct gct cgt cct ttg gca 288 Ser Ser Pro Pro Ser Ala Ser Met Arg Leu Ser Ala Arg Pro Leu Ala 85 90 95

aaa cat gtc tct ccc ctt ctc cgg gca aaa cag gct tct cct ttt cca 336 Lys His Val Ser Pro Leu Leu Arg Ala Lys Gln Ala Ser Pro Phe Pro 100 105 110

tagaaccagc agegggcctc tecatetect ttettggtcc aeegggttte gtteteettt 396

catccgtcaa tgcaggtttc atctcgtcct ttggggaaac atgtccctcc ccttctccgg 456

gcaaaacagg cttctccttt tccatagaac cagcagcggg cctctccatc tccgttqqtq 516

gtccaccgan respected tittcatctg tcaatgcagg tittcgtctcg tgctttggca 576

aaacatgtcc cramatict ccggggtg 604

<210> 83

<400>,8:

Pro Met G.: : · Val Ser Pro Ser Pro Phe Val Gln Ser Asp Ser Pro 1 10 15

Ser Ser I: In Gln Ser Ala Ser Pro Pro Pro Ser Glu Tyr Gln
25 30

Asp Sc: : . . : Pro Leu Ala Glu Ser Val Ser Ser Leu Pro Leu 40 45

Ala Lyc .. • ·: Fro Leu His Leu Thr Gln His Pro Ser Pro Leu 50 55 60

Leu Trr ::: : Ala Ser Pro Ser Pro Phe Leu Val Gln Arg Asp 65 70 75 80

Ser Ser :: : : Ala Ser Met Arg Leu Ser Ala Arg Pro Leu Ala . 90 95

Lys Hir ... : Leu Leu Arg Ala Lys Gln Ala Ser Pro Phe Pro 105 110

<210> %; <211> %; <212: Th: <213> T - Sdri

ggtttgt " aaaaaatgtc cttccccttc tccgggcaac acaagcttgt 120

cetttte .. :: gegggetttt cateteeegt tggtggteea eegggttteg 180

ccattetegg caaaacaage ttgteette ccatagaace ageagegge etetecateg 300 ccattetegg tecacegggt ttegttete ttteateegt caatgeaggt ttegtetegt 360 cctttggcaa aacatgtete teceettete egggcaaaac aggettetee ttttecatag 420 aaceageage gggeetetee ateteette ttggteeace gggtttegtt etettteat 480 ccgteaatge aggtttegte tegteettag geaaaacatg teteteeet teteegggea 540 acacaageg 549

<210> 85 <211> 270 <212> DNA <213> Toxoplasma gondii <220> <221> CDS

<222> (1)..(270)

aca gac tcg aag tca aca aaa gca aac tca gcg gca gag tgc cag cag 96
Thr Asp Ser Lys Ser Thr Lys Ala Asn Ser Ala Ala Glu Cys Gln Gln
20 25 30

atg tgc ctc aac gat gag agg tgt gac ttt ttc acg tgg caa cag gcg $\,$ 144 Met Cys Leu Asn Asp Glu Arg Cys Asp Phe Phe Thr Trp Gln Gln Ala $\,$ 35 $\,$ 40 $\,$ 45

ggc aag cat tgt tgg ttt aag gct ggg gcg tcc act gcc tca aca aaa 192 Gly Lys His Cys Trp Phe Lys Ala Gly Ala Ser Thr Ala Ser Thr Lys 50 55 60

tac aat cgg gct ggc gac tat tct gca cca aaa cac tgc ggc ctg ccg 240 Tyr Asn Arg Ala Gly Asp Tyr Ser Ala Pro Lys His Cys Gly Leu Pro 65 70 75 80

acc aca tgt gtc aag gag cgg acc aag tcg

Thr Thr Cys Val Lys Glu Arg Thr Lys Ser

85

90

<210> 86

<211> 90

<212> PRT

<213> Toxoplasma gondii

<400> 86

Arg Thr Asp Glu His Trp Cys Ile Met Lys Asp Ile Gly Tyr Lys Gly
1 5 10 15

Thr Asp Ser Lys Ser Thr Lys Ala Asn Ser Ala Ala Glu Cys Gln Gln 20 25 30

Met Cys Leu Asn Asp Glu Arg Cys Asp Phe Phe Thr Trp Gln Gln Ala 35 40 45

Gly Lys His Cys Trp Phe Lys Ala Gly Ala Ser Thr Ala Ser Thr Lys
50 55 60

Tyr Asn Arg Ala Gly Asp Tyr Ser Ala Pro Lys His Cys Gly Leu Pro 65 70 75 80

Thr Thr Cys Val Lys Glu Arg Thr Lys Ser 85 90

<210> 87

<211> 306

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(306)

<400> 87

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Arg Arg Gln Gln Met Gly Pro Val Arg Ala Pro Asp Leu Gln Phe Asn
1 5 10 15

cag tcg cca ctg ctc ccc cac aac ctc ggc cct gcc cac gtt ccc atg 96 Gln Ser Pro Leu Leu Pro His Asn Leu Gly Pro Ala His Val Pro Met 20 25 30

gga ggt ctc ccg tcg cat cct cat atc tcg gac ttt cat aac tca tcg 144
Gly Gly Leu Pro Ser His Pro His Ile Ser Asp Phe His Asn Ser Ser
35 40 45

gag tcg cgc ccg caa cat ccg ctg ctt gcc agc ggg ctc gca tcg aga 192

Glu Ser Arg 1:0 Gln His Pro Leu Leu Ala Ser Gly Leu Ala Ser Arg 55 ctc gga 'a: :: ctg acg ccc cag gag aga cag ttc gtg ctc tct caa 240 Leu Gly 31: 41, Leu Thr Pro Gln Glu Arg Gln Phe Val Leu Ser Gln 70 75 288 Gln Ser Giv ., Ser Thr Ser Phe Leu Leu Pro Ala Leu Pro Ser Leu ₽5 90 toa gad with the gog 306 Ser Glu Ant. ... Der Ala <210> 8-<211> :. <212> PF: <400 - --Arg Ar: \cdot ": Gly Pro Val Arg Ala Pro Asp Leu Gln Phe Asn Gln Ser Pro His Asn Leu Gly Pro Ala His Val Pro Met 25 40 45 Glu S-· · :: His Pro Leu Leu Ala Ser Gly Leu Ala Ser Arg 55 60 Leu G! . Thr Pro Gln Glu Arg Gln Phe Val Leu Ser Gln 65 70 75 : Thr Ser Phe Leu Leu Pro Ala Leu Pro Ser Leu Gln : :90 Ser Gi. : Ala <210> - -<211> -<212> ::.

<220> <221> CDS <222> (1)..(804) <400> 89 cgc gga ggc att tca gtt ccc aca ctt tcc atc atg aat cag agc acc 48 Arg Gly Gly Ile Ser Val Pro Thr Leu Ser Ile Met Asn Gln Ser Thr 1 10 att gtt gcg acg tct gtg gtc gct ccg cag agc gca gtc tca ctt tcg Ile Val Ala Thr Ser Val Val Ala Pro Gln Ser Ala Val Ser Leu Ser 20 25 agg gcc cct agc cga cca ggg cct agc gag agt ttc ggt aaa cag caa 144 Arg Ala Pro Ser Arg Pro Gly Pro Ser Glu Ser Phe Gly Lys Gln Gln 35 40 gaa agt cgt cca ggt gtt tcg ggt gct ggc ctc gct gaa agc aaa cgc 192 Glu Ser Arg Pro Gly Val Ser Gly Ala Gly Leu Ala Glu Ser Lys Arg 55 gtg ccc agc ctt act cag ccg tct ctg gaa cgg tcc gta acc ata tca 240 Val Pro Ser Leu Thr Gln Pro Ser Leu Glu Arg Ser Val Thr Ile Ser 65 70 75 cga cgc aaa att gat gcg gtg ggc atg tca ctc gtg ccg aag tta gac 288 Arg Arg Lys Ile Asp Ala Val Gly Met Ser Leu Val Pro Lys Leu Asp agg aca acg act tot ott gca gcg aag gag gag aaa tto agt tot atc 336 Arg Thr Thr Ser Leu Ala Ala Lys Glu Glu Lys Phe Ser Ser Ile 100 105 110 gac aag ata gtc tca aag cca acc cat tct ttt ggg gag agt tcc aaa 384 Asp Lys Ile Val Ser Lys Pro Thr His Ser Phe Gly Glu Ser Ser Lys 115 120 tta cca gcg ggt ata atg aaa gcg aaa tca atg ttt ccg tca caa acc 432 Leu Pro Ala Gly Ile Met Lys Ala Lys Ser Met Phe Pro Ser Gln Thr 130 135 ctt tcc gca ccg tgg aac gct cct gct cgt tgc gct cgg aaa gac agc 480 Leu Ser Ala Pro Trp Asn Ala Pro Ala Arg Cys Ala Arg Lys Asp Ser 145 150 155 160 ttc ggg acg aag gcc tgg atc gaa aaa ctg caa aga gaa acc aca gac 528 Phe Gly Thr Lys Ala Trp Ile Glu Lys Leu Gln Arg Glu Thr Thr Asp

165 170 175

acc tcg cag cct cca ctt gag cgt caa aag tcg cag cgc ctc gcg caa 576
Thr Ser Gln Pro Pro Leu Glu Arg Gln Lys Ser Gln Arg Leu Ala Gln
180 185 190

acc gag cct gtg cag aaa ctc aag aca tcc tgg ttg gag cct cct caa 624
Thr Glu Pro Val Gln Lys Leu Lys Thr Ser Trp Leu Glu Pro Pro Gln
195 200 205

gag gtc gaa agt gga cat gga gtc gct gaa ggc gac gat ctc agc gtt 672 Glu Val Glu Ser Gly His Gly Val Ala Glu Gly Asp Asp Leu Ser Val 210 215 220

gca gca gcc gag tat cac gtc cca gaa acg gaa gat gga aaa ccc agc 720 Ala Ala Ala Glu Tyr His Val Pro Glu Thr Glu Asp Gly Lys Pro Ser 225 230 240

ttc aaa cct agc gac ccc cgc gtg tgg aat cgc gag tgg atc cac cga 768
Phe Lys Pro Ser Asp Pro Arg Val Trp Asn Arg Glu Trp Ile His Arg
245 250 255

agg ata cat aac ccc gtc ctc agt cgc tcg aac cgg
Arg Ile His Asn Pro Val Leu Ser Arg Ser Asn Arg
260 265

<210> 90

<211> 268

<212> PRT

<213> Toxoplasma gondii

<400> 90

Arg Gly Gly Ile Ser Val Pro Thr Leu Ser Ile Met Asn Gln Ser Thr 1 5 10 15

Ile Val Ala Thr Ser Val Val Ala Pro Gln Ser Ala Val Ser Leu Ser 20 25 30

Arg Ala Pro Ser Arg Pro Gly Pro Ser Glu Ser Phe Gly Lys Gln Gln 35 40 45

Glu Ser Arg Pro Gly Val Ser Gly Ala Gly Leu Ala Glu Ser Lys Arg
50 55 60

Val Pro Ser Leu Thr Gln Pro Ser Leu Glu Arg Ser Val Thr Ile Ser 65 70 75 80

Arg Arg Lys Ile Asp Ala Val Gly Met Ser Leu Val Pro Lys Leu Asp 85 90 95

Arg Thr Thr Ser Leu Ala Ala Lys Glu Glu Lys Phe Ser Ser Ile 100 105 110

Asp Lys Ile Val Ser Lys Pro Thr His Ser Phe Gly Glu Ser Ser Lys 115 120 125

Leu Pro Ala Gly Ile Met Lys Ala Lys Ser Met Phe Pro Ser Gln Thr 130 135 140

Phe Gly Thr Lys Ala Trp Ile Glu Lys Leu Gln Arg Glu Thr Thr Asp 165 170 175

Thr Ser Gln Pro Pro Leu Glu Arg Gln Lys Ser Gln Arg Leu Ala Gln 180 185 190

Thr Glu Pro Val Gln Lys Leu Lys Thr Ser Trp Leu Glu Pro Pro Gln 195 200 205

Glu Val Glu Ser Gly His Gly Val Ala Glu Gly Asp Asp Leu Ser Val 210 215 220

Ala Ala Ala Glu Tyr His Val Pro Glu Thr Glu Asp Gly Lys Pro Ser 225 230 235 240

Phe Lys Pro Ser Asp Pro Arg Val Trp Asn Arg Glu Trp Ile His Arg 245 250 255

Arg Ile His Asn Pro Val Leu Ser Arg Ser Asn Arg 260 265

<210> 91

<211> 867

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(867)

<400> 91

cgg gat cca gct ggc aag gca gta aag gca gcc aca ggg ata cca 48

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Arç 1		Pro	Ala	Gly 5		Ala	Val	. Lys	Lys 10		a Ala	Thr	Gly	/ Ile		
aag Lys	cct Pro	gca Ala	gct Ala 20	cca Pro	ggt Gly	Gly	aag Lys	gca Ala 25	gtc Val	aag Lys	gtg Val	act Thr	cct Pro 30	Val	gcg Ala	96
cga Arg	aaa Lys	cct Pro 35	gtt Val	gca Ala	cca Pro	aag Lys	gca Ala 40	Ala	gct Ala	cca Pro	gac Asp	ggc Gly 45	aag Lys	gcg Ala	gtc Val	144
aag Lys	aag Lys 50	gca Ala	acc Thr	gta Val	gtc Val	gtg Val 55	cca Pro	aag Lys	cct Pro	gca Ala	gct Ala 60	ccc Pro	agt Ser	ggc Gly	aag Lys	192
gca Ala 65	gtg Val	aag Lys	aag Lys	ccg Pro	gtt Val 70	gtc Val	agc Ser	gtg Val	cca Pro	aag Lys 75	cct Pro	gca Ala	aca Thr	ctc Leu	ggt Gly 80	240
ggc Gly	aag Lys	gca Ala	gtg Val	aag Lys 85	aag Lys	cca Pro	gct Ala	gcc Ala	ggc Gly 90	gtg Val	cca Pro	aag Lys	ccc Pro	gca Alaʻ 95	gct Ala	288
ccc Pro	gat Asp	ggc Gly	aag Lys 100	gcg Ala	gtg Val	aga Arg	aag Lys	cca Pro 105	gtt Val	gtc Val	ggc Gly	gtg Val	cca Pro 110	aag Lys	ccc Pro	336
gca Ala	gct Ala	ccc Pro 115	gat Asp	ggt Gly	aag Lys	gcg Ala	gcg Ala 120	aaa Lys	aag Lys	cca Pro	gcg Ala	tcc Ser 125	ggc Gly	gtg Val	cca Pro	384
aag Lys	cct Pro 130	gcg Ala	gat Asp	cca Pro	Ala	ggc Gly 135	aag Lys	gca Ala	gta Val	aag Lys	aag Lys 140	gca Ala	gcc Ala	aca Thr	ggg ggg	432
ata Ile 145	cca Pro	aag Lys	cct Pro	Ala	gct Ala 150	cca Pro	ggt Gly	ggc Gly	aag Lys	gca Ala 155	atc Ile	aag Lys	gtg Val	Thr	cct Pro 160	480
gtc Val	gcg Ala	cga Arg	Lys	cct Pro 165	gtt Val .	gca Ala	cca Pro	Lys	gca Ala 170	gca Ala	gct Ala	cca Pro	Asp	ggc Gly 175	aag Lys	528
gca Ala	gtc Val	aag Lys	aag Lys 180	gca Ala	acc Thr	gta Val	Val	gtg Val 185	cca Pro	aag Lys	cct Pro	Ala	gct Ala 190	ccc Pro	agt Ser	576
ggc	aag	gca	gtg	aag	aag	сса	gtt	gtc	agc	gtg	сса	aag	cct	gca	acg	624

ctc gat ggc aag gcg gtg aga aag cca gtt gtc ggc gtg cca aag ccc 672 Leu Asp Gly Lys Ala Val Arg Lys Pro Val Val Gly Val Pro Lys Pro 210 215 220

gca gct ccc gat ggt aag gcg gtg aaa aag cca gtt gtc ggc gtg cca 720 Ala Ala Pro Asp Gly Lys Ala Val Lys Lys Pro Val Val Gly Val Pro 225 230 235 240

aag cct gca gct cca gat gac acg gga atc aac aag gcg acc ctt gtc 768 Lys Pro Ala Ala Pro Asp Asp Thr Gly Ile Asn Lys Ala Thr Leu Val 245 250 255

acg cgg aaa cct gag gct cca gac gtg aag gta gtc aag aag gca acc 816 Thr Arg Lys Pro Glu Ala Pro Asp Val Lys Val Val Lys Lys Ala Thr 260 265 270

gta gtt gtg cca aaa cct gaa gcg cca gat ata aag gta atg acg gat 864 Val Val Val Pro Lys Pro Glu Ala Pro Asp Ile Lys Val Met Thr Asp 275 280 285

ccg Pro

<210> 92

<211> 289

<212> PRT

<213> Toxoplasma gondii

<400> 92

Arg Asp Pro Ala Gly Lys Ala Val Lys Lys Ala Ala Thr Gly Ile Pro 1 5 10 15

Lys Pro Ala Ala Pro Gly Gly Lys Ala Val Lys Val Thr Pro Val Ala 20 25 30

Arg Lys Pro Val Ala Pro Lys Ala Ala Ala Pro Asp Gly Lys Ala Val 35 40 45

Lys Lys Ala Thr Val Val Val Pro Lys Pro Ala Ala Pro Ser Gly Lys 50 55 60

Ala Val Lys Lys Pro Val Val Ser Val Pro Lys Pro Ala Thr Leu Gly 65 70 75 80

Gly Lys Ala Val Lys Lys Pro Ala Ala Gly Val Pro Lys Pro Ala Ala 85 90 95

- Pro Asp Gly Lys Ala Val Arg Lys Pro Val Val Gly Val Pro Lys Pro 100 105 110
- Ala Ala Pro Asp Gly Lys Ala Ala Lys Lys Pro Ala Ser Gly Val Pro 115 120 125
- Lys Pro Ala Asp Pro Ala Gly Lys Ala Val Lys Lys Ala Ala Thr Gly 130 135 140
- Val Ala Arg Lys Pro Val Ala Pro Lys Ala Ala Ala Pro Asp Gly Lys 165 170 175
- Ala Val Lys Lys Ala Thr Val Val Val Pro Lys Pro Ala Ala Pro Ser 180 185 190
- Gly Lys Ala Val Lys Lys Pro Val Val Ser Val Pro Lys Pro Ala Thr 195 200 205
- Leu Asp Gly Lys Ala Val Arg Lys Pro Val Val Gly Val Pro Lys Pro 210 215 220
- Ala Ala Pro Asp Gly Lys Ala Val Lys Lys Pro Val Val Gly Val Pro 225 230 235 240
- Lys Pro Ala Ala Pro Asp Asp Thr Gly Ile Asn Lys Ala Thr Leu Val 245 250 255
- Thr Arg Lys Pro Glu Ala Pro Asp Val Lys Val Val Lys Lys Ala Thr 260 265 270
- Val Val Val Pro Lys Pro Glu Ala Pro Asp Ile Lys Val Met Thr Asp 275 280 285

Pro

<210> 93

<211> 1434

<212> DNA

<213> Toxoplasma gondii

<220> <221> CDS <222> (1)..(492) <400> 93 cgg ctt gtg ttg ccc gga gaa ggg gag aga cat gtt ttg cca aag gac Arg Leu Val Leu Pro Gly Glu Gly Glu Arg His Val Leu Pro Lys Asp 15 gag acg aaa cct gca ttg acg gat gaa aag aga acg aaa ccc ggt gga . 96 Glu Thr Lys Pro Ala Leu Thr Asp Glu Lys Arg Thr Lys Pro Gly Gly _ 20 25 cca agg aag gag atg gag agg ccc gct gct ggt tct atg gaa aag gac 144 Pro Arg Lys Glu Met Glu Arg Pro Ala Ala Gly Ser Met Glu Lys Asp 35 40 aag ctt gtt ttg ccc gga gaa ggg gag aga cat gtt ttg cca aag gac 192 Lys Leu Val Leu Prc Gly Glu Gly Glu Arg His Val Leu Pro Lys Asp 50 55 gag acg aaa cct gca ttg acg gag gaa aag aga acg aaa ccc ggt gga 240 Glu Thr Lys Pro Ala Leu Thr Glu Glu Lys Arg Thr Lys Pro Gly Gly cca cga acg gag atg gag agg ccc gct gct ggt tct atg gaa aag gac 288 Pro Arg Thr Glu Met Glu Arg Pro Ala Ala Gly Ser Met Glu Lys Asp 85 90 95 aag oct ggt ttg occ gga gaa ggg gag aga cat gtt ttg oca aag qac 336 Lys Pro Gly Leu Pro Gly Glu Gly Glu Arg His Val Leu Pro Lys Asp 100 105 gag acg aaa cct gca ttg acg gag gaa aag aga acg aac ctg gcg gac Glu Thr Lys Pro Ala Leu Thr Glu Glu Lys Arg Thr Asn Leu Ala Asp 115 120 125 caa gaa agg aga tgg aga gcc cgc tgc tgg ttc ttg gaa aag gag aac 432 Gln Glu Arg Arg Trp Arg Ala Arg Cys Trp Phe Leu Glu Lys Glu Asn 130 ctg ttt ggc ccg gag aag ggg aga gac acg ctt cgc caa agg acg aga 480 Leu Phe Gly Pro Glu Lys Gly Arg Asp Thr Leu Arg Gln Arg Thr Arg 150 155 cga aag ccg cat tgacgcaaaa ggaggtgacg aatcccgttg aaccaaqaaa 532 Arg Lys Pro His

ggcgatggag aggcccgctg ctggttctat ggaaaaggaa aacctgtttc cccggagaag 592 gggagggaca tgttttgcca aagcacagac gaaacctgca ttgacagatg aaaagagaac 652 gaaacccggt ggaccaccaa cggagatgga gaggcccgct gctggtttta tggaaaagga 712 gaageetgtt ttgeeeggag aaggggaggg acatgtttee eeaaaggaeg agatgaaace 772 tgcattgacg gatgaaaaga gaacgaaacc cggtggacca agaaaggaga tggagaggcc 832 cgctgctggt tttatggaaa aggagaagcc tgttttgccc ggagaagggg agagacatgt 892 tttgccaaag gacgagcaga aagccgcatt gacgcagaag gaggtgacga atcccgttga 952 accaagaaag gagatggaga ggcccgctgt gcccatagaa ggggagaagg gtgttgtgtc 1012 aagtgaagag gagaagcctg tttcgccaaa ggaagcgacg agacggattt tgccaaagga 1072 agggaaagag atttggtact cggaaggagg aggtgaagcc gattgtgcga agggcaaaga 1132 gggggagacg gattgcacaa aaggggaagg agaaacaaat tgcatcgaag gaggggaaga 1192 aacccgctgt accaaaggaa ggtgaggaaa gacccgctga accaacggaa ggcgaggaaa 1252 ggcccgttgg gccaaaggaa ggcgaggaaa gacccgttgt gccggacgta gacaaggaga 1312 aacctgttgt gcctgaagga gacaaggaga aacctgttgt gccggaagga gacaaggatc 1372 accetgette tgecagagea ggatgaggag aaacaegeta catgggagaa agaaatgate 1432 cg 1434

<210> 94

<211> 164

<212> PRT

<213> Toxoplasma gondii

<400> 94

Arg Leu Val Leu Pro Gly Glu Gly Glu Arg His Val Leu Pro Lys Asp
1 5 10 15

Glu Thr Lys Pro Ala Leu Thr Asp Glu Lys Arg Thr Lys Pro Gly Gly
20 25 30

Pro Arg Lys Glu Met Glu Arg Pro Ala Ala Gly Ser Met Glu Lys Asp 35 40 45

Lys Leu Val Leu Pro Gly Glu Gly Glu Lys Arg His Val Leu Pro Lys Asp 60

Glu Thr Lyb Fir Ala Leu Thr Glu Glu Lys Arg Thr Lys Pro Gly Gly 80

Pro Arg Tr: Ala Met Glu Arg Pro Ala Ala Gly Ser Met Glu Lys Asp 90

Lys Pro Gl, Iso Sic Gly Glu Gly Glu Arg His Val Leu Pro Lys Asp 105

Glu Thr La Sic Ala Leu Thr Glu Glu Lys Arg Thr Asn Leu Ala Asp 120

Glu Thr La Sic Ala Leu Thr Glu Glu Lys Arg Thr Asn Leu Ala Asp 120

Glu Glu Are Are Tre Arg Ala Arg Cys Trp Phe Leu Glu Lys Glu Asn 130

Leu Phe ... · . . Lys Gly Arg Asp Thr Leu Arg Gln Arg Thr Arg 145 150 155 160

Arg Lys i: "

<210> 9. <211> 0F <212> DW <213> T : ... ** ::ndii <220> <221> CU. <222> :

cga cc... ang gag cag cct gct gtt ccg cgg cag gaa gaa 48
Arg P: Arg Glu Gln Pro Ala Val Pro Arg Gln Glu Glu
1 10 15

gaa gae : : : t tta cca gaa aca ggg gcg aaa cat gtt tta 144
Glu As: Val Leu Pro Glu Thr Gly Ala Lys His Val Leu
40 45

ccg gaa a' aaa tcc act ttg acg cag aaa gag ctg aca aaa 192

***	, ,,,,	2000													101/0	
Pro	Glu 50	Ile	Ala	Thr	Glu	Ser 55	Thr	Leu	Thr	Gln	Lys 60	Glu	Leu	Thr	Lys	
													tct Ser			240
													gtc Val			288
													acg Thr 110			336
													atg Met			384
													ttg Leu			432
													aaa Lys			480
													atg Met	-	_	528
													ttg Leu 190		-	576
													aaa Lys			624
													atg Met			672
gac Asp 225	aag Lys	cg														680

<210> 96

<211> 226

<212> PRT

<213> Toxoplasma gondii

<400> 96

- Arg Pro Arg Ala Gly Arg Glu Gln Pro Ala Val Pro Arg Gln Glu Glu
 1 5 10 15
- Gln Lys Leu Val Leu Gln Lys Thr Glu Arg Lys Pro Val Leu Pro Glu 20 25 30
- Glu Asp Gln Lys Pro Val Leu Pro Glu Thr Gly Ala Lys His Val Leu 35 40 45
- Pro Glu Ile Ala Thr Glu Ser Thr Leu Thr Gln Lys Glu Leu Thr Lys 50 55 60
- Pro Val Glu Thr Arg Gln Asp Met Arg Gly Thr Ala Gly Ser Met Asp 65 70 75 80
- Glu Lys Lys Pro Val Leu Pro Gly Glu Trp Glu Arg His Val Leu Pro 85 90 95
- Lys Asp Glu Thr Lys Pro Ala Leu Thr Glu Glu Lys Arg Thr Lys Pro 100 105 110
- Val Glu Pro Arg Lys Glu Met Glu Arg Pro Ala Arg Pro Met Glu Glu 115 120 125
- Glu Lys Pro Val Leu Pro Gly Glu Gly Glu Arg His Val Leu Pro Lys 130 135 140
- Asp Gly Met Lys Pro Ala Leu Thr Asp Glu Lys Arg Thr Lys Pro Gly 145 150 155 160
- Gly Pro Arg Lys Glu Met Glu Arg Pro Ala Ala Gly Ser Met Glu Lys 165 170 175
- Asp Lys Leu Val Leu Pro Gly Glu Gly Glu Arg His Val Leu Pro Lys 180 185 190
- Asp Glu Thr Lys Pro Ala Leu Thr Asp Glu Lys Arg Thr Lys Pro Gly
 195 200 205
- Gly Pro Arg Lys Ala Met Glu Arg Pro Ala Ala Gly Ser Met Glu Lys 210 220 .

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Asp Lys
 225
<210> 6
<211> : ••
<212> DMA
<213> T & : . . . . . . gondii
<220>
<221> C:.:
<222> : :;
<400 . "
ccg qt : :: ;; ;;c gac ccc cgt ggc tgt tcg cag caa agc gga gac
                                                                 48
Pro Va. A: Amp Asp Pro Arg Gly Cys Ser Gln Gln Ser Gly Asp
                                                        15
acc add : . .: agt ccc gcc aca cct ggt ggt cgg ccg gct ggt
                                                                 96
Thr A:: ... Ser Pro Ala Thr Pro Gly Gly Arg Pro Ala Gly
ggg : . . . . . . gcg aca agc ccg aag gga cag gcc ttt gcc ccg
            ing Ala Thr Ser Pro Lys Gly Gln Ala Phe Ala Pro
                            40
cgg cgg cg; . . . . ::; gag ata aag ccc cag gag aca gga aac agt gga
                                                                 192
         .y Glu Ile Lys Pro Gln Glu Thr Gly Asn Ser Gly
Arg 31
    Ē
                        55
               ::; gga aag gaa gca agt gga gac gcg aac act tcg
gac a:
                                                                 240
Asp ...:
             . .u Gly Lys Glu Ala Ser Gly Asp Ala Asn Thr Ser
                    70
                                        75
               ··: tcg ggc gaa gtg gac aag aca gcc gag gtg gag
gaa ⊹::
                                                                 288
Glu ´
                . Ser Gly Glu Val Asp Lys Thr Ala Glu Val Glu
                                    90
aca :
                                                                 296
Thr A...
<210
<211 ·
<212 · ! .
<213 ·
                :>ndii
<400>
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Pro Val Asp Val Asp Asp Pro Arg Gly Cys Ser Gln Gln Ser Gly Asp 1 5 10 15

Thr Arg Asp Ser Ser Pro Ala Thr Pro Gly Gly Arg Pro Ala Gly 20 25 30

Gly Ala Gly Gly Ala Ala Thr Ser Pro Lys Gly Gln Ala Phe Ala Pro 35 40 45

Arg Gly Glu Gly Glu Ile Lys Pro Gln Glu Thr Gly Asn Ser Gly 50 55 60

Asp Ser Lys Ala Glu Gly Lys Glu Ala Ser Gly Asp Ala Asn Thr Ser 65 70 75 80

Glu Gly Lys Arg Leu Ser Gly Glu Val Asp Lys Thr Ala Glu Val Glu 85 90 95

Thr Ala

<210> 99

<211> 723

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(159)

<400> 99

cga tcc tcc cga ggg acc gca gga agg ctc gcg tcc gaa gaa gac gac 48 Arg Ser Ser Arg Gly Thr Ala Gly Arg Leu Ala Ser Glu Glu Asp Asp 1 5 10 15

aga gaa gac ggg gaa gac gca ggc tct agg cgt cga gag aag gac ttc 144 Arg Glu Asp Gly Glu Asp Ala Gly Ser Arg Arg Arg Glu Lys Asp Phe 35 40 45

ttc cca gac acg act tgaatgcgta aaggcgtatt tttgtttccg atgaaaactc 199 Phe Pro Asp Thr Thr 50

gacaggggaa gcgacttctc gcctctgagg aatccgacag tgacggagg aaggggaag 259
gagacgcaga gaaggacgcg tcaggaggat ccggaattcc ggatcgggcg atggccccgg 319
agcgcgtgaag ggcggtacac tgaagaacca acggaagaac actgggggtc gaaaatgtgt 379
ttcctttccg atgtggtctt cccagctttc ctgcagacat gtgtacagaa cagctgagaa 439
aaaacgacga aagctccaat tgtctcttcg ttctcgagca gagaaaaccc cccgaggcct 499
tcgcttggtc agggcgaaac ctcaagggtg catgcagagt cggccgtgcc cagagtagcc 559
tagtcatgca gcccatcagt agcttaattt gacgcaatgg ctattttac attgtgaaga 619
gggttttcca atcaacaac gccagagaag cctgtgttct ggaaaacctg aacgacggcc 679
gtcgttcccc tgtctgctt acccctgac agtgcgtggt gagg 723

<210> 100

<211> 53

<212> PRT

<213> Toxoplasma gondii

<400> 100

Arg Ser Ser Arg Gly Thr Ala Gly Arg Leu Ala Ser Glu Glu Asp Asp 1 5 10 15

Gly Asp Asn Glu Glu Glu Glu Glu Glu Glu Arg Glu Arg Glu
20 25 30

Arg Glu Asp Gly Glu Asp Ala Gly Ser Arg Arg Arg Glu Lys Asp Phe
35 40 45

Phe Pro Asp Thr Thr 50

<210> 101

<211> 270

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(270)

<400> 101

WO 99/32633 PCT/US98/27137 cgg aag ccg att gtg cga agg gca aag agg ggg aga cgg att gca caa 48 Arg Lys Pro Ile Val Arg Arg Ala Lys Arg Gly Arg Arg Ile Ala Gln 10 aag ggg aag gag aaa caa att gca tcg aag gag ggg aag aaa ccc gct Lys Gly Lys Glu Lys Gln Ile Ala Ser Lys Glu Gly Lys Lys Pro Ala 20 25 gta cca aag gaa ggt gag gaa aga ccc gct gaa cca acg gaa ggc gag 144 Val Pro Lys Glu Gly Glu Glu Arg Pro Ala Glu Pro Thr Glu Gly Glu 35 gaa agg ccc gtt ggg cca aag gaa ggc gag gaa aga ccc gtt gtg ccg 192 Glu Arg Pro Val Gly Pro Lys Glu Gly Glu Glu Arg Pro Val Val Pro 50 55 60 gac gta gac aag gag aaa cct gtt gtg cct gaa gga gac aag gag aaa 240 Asp Val Asp Lys Glu Lys Pro Val Val Pro Glu Gly Asp Lys Glu Lys 65 70 cct gtt gtg ccg gaa gga gac aag gat ccg 270 Pro Val Val Pro Glu Gly Asp Lys Asp Pro 85 <210> 102 <211> 90 <212> PRT <213> Toxoplasma gondii <400> 102 Arg Lys Pro Ile Val Arg Arg Ala Lys Arg Gly Arg Arg Ile Ala Gln Lys Gly Lys Glu Lys Gln Ile Ala Ser Lys Glu Gly Lys Lys Pro Ala 25 Val Pro Lys Glu Gly Glu Glu Arg Pro Ala Glu Pro Thr Glu Gly Glu 35 Glu Arg Pro Val Gly Pro Lys Glu Gly Glu Glu Arg Pro Val Val Pro 50 55 Asp Val Asp Lys Glu Lys Pro Val Val Pro Glu Gly Asp Lys Glu Lys 65 70 80 Pro Val Val Pro Glu Gly Asp Lys Asp Pro 85 90

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<210> 103
 <211> 503
 <212> DNA
 <213> Toxoplasma gondii
 <220>
 <221> CDS
 <222> (1)..(186)
 <400> 103
cgg cat ctc tgg tgc gtg cgc gag aga tcc ccg caa cga gaa aga tgg
Arg His Leu Trp Cys Val Arg Glu Arg Ser Pro Gln Arg Glu Arg Trp
                                    10
                                                       15
age ttc gtc tcg ttc tcg ctt ttc tct ttc cag ttc ttt ttc age
                                                               96
Ser Phe Val Ser Phe Ser Leu Phe Phe Ser Phe Gln Phe Phe Ser
             20
                                25
aag caa gtc tcg cgc ctc cct cgt ccg agc agc gtc act gca ctg tgg
Lys Gln Val Ser Arg Leu Pro Arg Pro Ser Ser Val Thr Ala Leu Trp
                            40
                                               45
186
Ala Ile Ser Arg Lys Lys Ala Lys Lys Arg Asp Asp Gly Arg
     50
                        55
                                           60
taatggcgcg aaaatctatc ccaaaaacac atatatgcct tatggcagtg agcgaagaga 246
gggaactgcc aacgccttgg cggaagcccg ttctccaaac gaggttgagg taccaaacct 306
gcatgcggag agaccaaggc aggttttgtc ttccgtcgct tccgtggatg cttttcgcac 366
gtatgcaaaa gagagaacgg gaccaagtgc aagaagttat agagcagtcc cgacgacaga 426
gacgcancta gaggccgagc aagaatcgtt tttttcttct cgtaagggaa acgcagtgca 486
tanaagcaaa agaccgg
                                                              503
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<210> 104

<211> 62

<212> PRT

<213> Toxoplasma gondii

<400> 104

Arg His Leu Trp Cys Val Arg Glu Arg Ser Pro Gln Arg Glu Arg Trp

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W	O 99/3	2633													PCT/	US98/27137
1				5					10					15		
Ser	Phe	Val	Ser 20	Phe	Ser	Leu	Phe	Phe 25	Ser	Phe	Gln	Phe	Phe 30	Phe	Ser	
Lys	Gln	Val 35	Ser	Arg	Leu	Pro	Arg 40	Pro	Ser	Ser	Val	Thr 45	Ala	Leu	Trp	
Ala	Ile 50	Ser	Arg	Lys	Lys	Ala 55	Lys	Lys	Arg	Asp	Asp 60	Gly	Arg			
<212 <212	0> 10 1> 32 2> Di 3> To	22 JA	lasma	a gor	ndii											
	L> C[(219)													
<400)> 10)5														
												gtg Val				48
	_		_									ggg G1 v				96

Leu Ala Gly Gly Gln Arg Arg Cys Asn Glu Ser Arg Gly Met Glu Glu
20 25 30

gcg agg aaa agg agg tgt ctc gcg atg cgg tgc cag tgg act tcn tct $\,$ 144 Ala Arg Lys Arg Arg Cys Leu Ala Met Arg Cys Gln Trp Thr Xaa Ser $\,$ 35 $\,$ 40 $\,$ 45

gcg cta gat tgg agg gag agc tgg aaa aat gcc gag aca gct tcg cac 192 Ala Leu Asp Trp Arg Glu Ser Trp Lys Asn Ala Glu Thr Ala Ser His 50 55 60

gtc aca ttc ccg acg aaa cgc ccg cca tgaaggaaat cacagacatc 239
Val Thr Phe Pro Thr Lys Arg Pro Pro

accaacctic ccgccgtggc taaaggaccg tcctgtgtat gtacagtttt tccaggcgaa 299

agccgaagag acagcgaaac cgg 322

<210> 106

<211> 73

<212> PRT

<213> Toxoplasma gondii

<400> 106

Arg Arg Asp Leu Arg Thr Ser Val Trp Asp Ala Arg Val Tyr Val His 1 5 10 15

Leu Ala Gly Gly Gln Arg Arg Cys Asn Glu Ser Arg Gly Met Glu Glu 20 25 30

Ala Arg Lys Arg Arg Cys Leu Ala Met Arg Cys Gln Trp Thr Xaa Ser 35 40 45

Ala Leu Asp Trp Arg Glu Ser Trp Lys Asn Ala Glu Thr Ala Ser His 50 55 60

Val Thr Phe Pro Thr Lys Arg Pro Pro 65 70

<210> 107

<211> 390

<212> DNA

<213> Toxoplasma gondii

<220>

<223> N = unknown at 104

<220>

<223> Xaa = unknown at 35

<220>

<221> CDS

<222> (1)..(201)

<400> 107

cgg cga atc ccc cag gaa ttg ttg aaa cag agt ctc aga ttc tac gga 48
Arg Arg Ile Pro Gln Glu Leu Leu Lys Gln Ser Leu Arg Phe Tyr Gly
1 5 10 15

ctc cga ggg cct ctg ctt gcc cgc cct gtg cac agg cgt cag cac gtg 96 Leu Arg Gly Pro Leu Leu Ala Arg Pro Val His Arg Arg Gln His Val 20 25 30

gtt ctc ana gaa aaa gtt ggt aag tgg aag tgg tgg agc caa gaa aaa 14 Val Leu Xaa Glu Lys Val Gly Lys Trp Lys Trp Trp Ser Gln Glu Lys 35 40 45

ctc aac tot tot tgt ttt ccg gag aat ttt cct ggt gtt caa ttc cac Leu Asn Ser Ser Cys Phe Pro Glu Asn Phe Pro Gly Val Gln Phe His 50 55 ggt tct gga tagtctttgt tgtattaaaa cacatctaga aggactgaga 241 Gly Ser Gly 65 cgttgtcggt agttgaatta cagacacttc gttttccagc gtcagcttgc atgcccgtcc 301 cctgtttctg gaacacaagc tttgagaagg aaacgagaca gagaacgacg aaggaagtga 361 agcaaatcct ctgacggatt tccattcgg 390 <210> 108 <211> 67 <212> PRT <213> Toxoplasma gondii <400> 108 Arg Arg Ile Pro Gln Glu Leu Leu Lys Gln Ser Leu Arg Phe Tyr Gly 10 Leu Arg Gly Pro Leu Leu Ala Arg Pro Val His Arg Arg Gln His Val 20 25 30 Val Leu Xaa Glu Lys Val Gly Lys Trp Lys Trp Ser Gln Glu Lys 35 40 45 Leu Asn Ser Ser Cys Phe Pro Glu Asn Phe Pro Gly Val Gln Phe His 50 55 Gly Ser Gly 65 <210> 109 <211> 699 <212> DNA <213> Toxoplasma gondii <220> <221> CDS <222> (1)..(699) <400> 109

WO 99/32633 PCT/US98/2713 ccg tgc gtc tgt gag gaa aag tgc aag aca ggg ccg aac tgt gac cag 48														US98/27137		
ccg Pro 1	tgc Cys	gtc Val	tgt Cys	gag Glu 5	Glu	aag Lys	tgc Cys	aag Lys	aca Thr 10	Gly	ccg Pro	aac Asn	tgt Cys	gac Asp 15	cag Gln	48
cat His	aaa Lys	ccg Pro	gag Glu 20	tgc Cys	tgt Cys	ggg Gly	tcg Ser	aac Asn 25	gac Asp	gac Asp	tgc Cys	cat His	cag Gln 30	cct Pro	cag Gln	96
Gly	tac Tyr	tgc Cys 35	aag Lys	atg Met	gac Asp	atg Met	tcc Ser 40	aca Thr	tgc Cys	atc Ile	tgc Cys	cgt Arg 45	cca Pro	ggc Gly	ttc Phe	144
acg Thr	ggc Gly 50	gag Glu	aac Asn	tgc Cys	gga Gly	aca Thr 55	cgg Arg	gaa Glu	gat Asp	ctg Leu	tgc Cys 60	gca Ala	ggt Gly	gtg Val	acg Thr	192
tgc Cys 65	aag Lys	aac Asn	ggc Gly	Gly	aca Thr 70	tgc Cys	gac Asp	tcc Ser	gtc Val	act Thr 75	ggc Gly	ctg Leu	tgc Cys	cag Gln	tgc Cys 80	240
														cac His 95		288
														acg Thr		336
														tgc Cys		384
Ser	agc Ser 130	gaa Glu	ggc Gly	aag Lys	tgc Cys	agc Ser 135	ggc Gly	aag Lys	acc Thr	tgc Cys	ttg Leu 140	agt Ser	gga Gly	cac His	tgc Cys	432
														ggc Gly		480
aga Arg	tgc Cys	gaa Glu	acg Thr	ctc Leu 165	gtc Val	aag Lys	gac Asp	tgc Cys	tgt Cys 170	gtt Val	gtg Val	aac Asn	gac Asp	acg Thr 175	tgc Cys	528
aag Lys	ttc Phe	ccc Pro	aac Asn 180	ggc Gly	gtc Val	tgc Cys	act Thr	gac Asp 185	agc Ser	aac Asn	agg Arg	tgt Cys	gag Glu 190	tgc Cys	cag Gln	576

WO 99/32633 PCT/US98/27137 ago ggo tạq gho cág ggo gao tạc ago aaa coa gto gao aag tạc gaa Ser Gly Trr Gly Gln Gly Asp Cys Ser Lys Pro Val Asp Lys Cys Glu 195 200 gac gtc agt :: auc aac ggt tca tca tgc gac gcg gac tcc ggc aca 672 Asp Val Se: Cro Ann Ash Gly Ser Ser Cys Asp Ala Asp Ser Gly Thr 210 215 220 tgc att tgr crr : a ggc ttt gga gac 699 Cys Ile Cy: i: Fire its Gly Phe Gly Asp 225 230 <210> 116 <211> 233 <212> FFT <213> Toxo; ndii <400> 11. ... ilu Lys Cys Lys Thr Gly Pro Asn Cys Asp Gln Pro Cys V... 10 25 Gly Tyr T. Amp Met Ser Thr Cys Ile Cys Arg Pro Gly Phe 40 Thr Gly ... A : 3ly Thr Arg Glu Asp Leu Cys Ala Gly Val Thr 50 55 Cys Lys A : . Thr Cys Asp Ser Val Thr Gly Leu Cys Gln Cys 65 ²0 75 Asp Ala : Y Lys Thr Cys Glu Ile Thr Lys Glu His Cys Cys Ii. : Acp Cys Asn Gly His Gly Thr Cys Asn Thr Ser 105 110 Asn Asn : ្ទ Glu Ala Gly Phe Ala Gly Thr Asn Cys Ser 120 Ser Ser .

135

150

ys Ser Gly Lys Thr Cys Leu Ser Gly His Cys

155

Ala Cys Val Cys Asp Pro Cys His Thr Gly Glu

140

160

130

145

Asn Pro A..

Arg Cys Glu Thr Leu Val Lys Asp Cys Cys Val Val Asn Asp Thr Cys Lys Phe Pro Asn Gly Val Cys Thr Asp Ser Asn Arg Cys Glu Cys Gln 180 185 Ser Gly Trp Gly Gln Gly Asp Cys Ser Lys Pro Val Asp Lys Cys Glu 195 200 Asp Val Ser Cys Asn Asn Gly Ser Ser Cys Asp Ala Asp Ser Gly Thr 210 215 220 Cys Ile Cys Pro Pro Gly Phe Gly Asp 225 230 <210> 111 <211> 419 <212> DNA <213> Toxoplasma gondii <220> <221> CDS <222> (1)..(417) <400> 111 gag atg agc gcc cca gat agg caa aca gga aag ctt tcc gat tta ccg 48 Glu Met Ser Ala Pro Asp Arg Gln Thr Gly Lys Leu Ser Asp Leu Pro 10 cca ttt gct gag ctg cca cag ctg gca gaa ata cca aag ctc tcc gaa 96 Pro Phe Ala Glu Leu Pro Gln Leu Ala Glu Ile Pro Lys Leu Ser Glu 20 ctt ccg aaa atc gcg gac atg ccg aaa ttt tcg gat atg ccc aag atg Leu Pro Lys Ile Ala Asp Met Pro Lys Phe Ser Asp Met Pro Lys Met 35 40 gcc gag atg ccc aag tta tca gat ata ccc aag atg gct gag atg ccc 192 Ala Glu Met Pro Lys Leu Ser Asp Ile Pro Lys Met Ala Glu Met Pro 50 55 aag tta tca gat ata ccc aag atg gct gag atg ccc aag tta tca gat 240 Lys Leu Ser Asp Ile Pro Lys Met Ala Glu Met Pro Lys Leu Ser Asp 70 ata ccc aag atg gct gag atg ccc aag ttt tca gat ata ccc aag atg 288

Ile Pro Lys Met Ala Glu Met Pro Lys Phe Ser Asp Ile Pro Lys Met 85 90 95

gct gag atg cca aag tta tca gat atg ccc. aga atg gct gac att cca 336 Ala Glu Met Pro Lys Leu Ser Asp Met Pro Arg Met Ala Asp Ile Pro 100 105 110

cag ttt cca gag atg cct agg atg gtt gac atg cct cag ttt cca gaa 384 Gln Phe Pro Glu Met Pro Arg Met Val Asp Met Pro Gln Phe Pro Glu 115 120 125

atc ccc agg atg gct gat atg ccg caa ttt ccg cg

Ile Pro Arg Met Ala Asp Met Pro Gln Phe Pro

130

135

<210> 112

<211> 139

<212> PRT

<213> Toxoplasma gondii

<400> 112

Glu Met Ser Ala Pro Asp Arg Gln Thr Gly Lys Leu Ser Asp Leu Pro 1 5 10 15

Pro Phe Ala Glu Leu Pro Gln Leu Ala Glu Ile Pro Lys Leu Ser Glu 20 25 30

Leu Pro Lys Ile Ala Asp Met Pro Lys Phe Ser Asp Met Pro Lys Met 35 40 45

Ala Glu Met Pro Lys Leu Ser Asp Ile Pro Lys Met Ala Glu Met Pro 50 55 60

Lys Leu Ser Asp Ile Pro Lys Met Ala Glu Met Pro Lys Leu Ser Asp 65 70 75 80

Ile Pro Lys Met Ala Glu Met Pro Lys Phe Ser Asp Ile Pro Lys Met 85 90 95

Ala Glu Met Pro Lys Leu Ser Asp Met Pro Arg Met Ala Asp Ile Pro 100 105 110

Gln Phe Pro Glu Met Pro Arg Met Val Asp Met Pro Gln Phe Pro Glu 115 120 125

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<210> 113
<211> 3: -
<212 - 1.5.
<2135 T x ; . . ma gondii
<220 ·
<221 · ::.
<400 · 11
gac a_{n+1} : • • • cct ctc ttt gga gca aac ggt gga acc tca gtt cgg
Asp G. . ... . Fro Leu Phe Gly Ala Asn Gly Gly Thr Ser Val Arg
      · :. Ego ago gto otg ott gtt oto gaa ooc goa gag ooc
                                                                 96
Leu L.: . . : Arg Ser Val Leu Leu Val Leu Glu Pro Ala Glu Pro
                                 25
ctg ~'..' : 79 ccc cac ccg ggg aga aga gac act ttt ctt gaa
                                                                 144
Leu : · · :rp Pro His Pro Gly Arg Arg Asp Thr Phe Leu Glu
                             40
             : 142 atc eeg tet eet tea tet egg eeg agt ege geg
ggc : ..
        .. Thy Ile Pro Ser Pro Ser Ser Arg Pro Ser Arg Ala
Gly 7. :
                         55
         egg aga etc tec acg att egg tet ett gec agg gat
                                                                 240
Alā i :
             · Thr Arg Leu Ser Thr Ile Arg Ser Leu Ala Arg Asp
 65
                     70
gga : : : * * * * * cc gag ctg gcg ggg gga ccg cag gaa aga gaa agt
                                                                 288
                ..r Glu Leu Ala Gly Gly Pro Gln Glu Arg Glu Ser
Gly ...
                ٠.,
                                    90
                                                        95
gt^
             19
                                                                 303
Val . ·
              1:0
<21
<211
<21:
<217
             -. :ondii
< 400
Asp G. . . . !ro Leu Phe Gly Ala Asn Gly Gly Thr Ser Val Arg
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1 5 10 15

Leu Ser Leu Asp Arg Ser Val Leu Leu Val Leu Glu Pro Ala Glu Pro
20 25 30

Leu Leu Ser Ser Trp Pro His Pro Gly Arg Arg Asp Thr Phe Leu Glu
35 40 45

Gly Asp Gly Ala Gly Ile Pro Ser Pro Ser Ser Arg Pro Ser Arg Ala
50 55 60

Ala Asp His Tyr Thr Arg Leu Ser Thr Ile Arg Ser Leu Ala Arg Asp 65 70 75 80

Gly Glu Val Asp Ser Glu Leu Ala Gly Gly Pro Gln Glu Arg Glu Ser 85 90 95

Val Arg Val Asp Pro 100

<210> 115

<211> 696

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(696)

<400> 115

cgc ggt aac gaa aaa aca tgc tca gat gcc aag cat cca gtg tac atc 48
Arg Gly Asn Glu Lys Thr Cys Ser Asp Ala Lys His Pro Val Tyr Ile
1 5 10 15

aaa ctt ggc aaa ggg gaa cgc gag gcc gta ttc aag tgt ggc gac ggc 96 Lys Leu Gly Lys Gly Glu Arg Glu Ala Val Phe Lys Cys Gly Asp Gly 20 25 30

ctc act act ctt gag cca tcg cag aac aca gat aaa cca aaa ttc tgt 144 Leu Thr Thr Leu Glu Pro Ser Gln Asn Thr Asp Lys Pro Lys Phe Cys 35 40 45

gaa tcg ata gac tgc aac gat act gca gaa ctt gaa aca acg ttc cca 192 Glu Ser Ile Asp Cys Asn Asp Thr Ala Glu Leu Glu Thr Thr Phe Pro 50 60

ggg gcg tac tgg gac gag aga aac aaa aga gcg aat ata tac aga ctg 240

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Gly 65	Ala	Tyr	Trp	Asp	Glu 70	Arg	Asn	Lys	Lys	Ala 75	Asn	Ile	Tyr	Arg	Leu 80	
_		cct Pro			-	-		_			_				-	288
		act Thr														336
		aca Thr 115			_	-	-		-		-		_		~	384
-		ggc Gly			-		-		-				-			432
		aag Lys	-					_	,	_						480
-		ccg Pro		-		-	-		-			_	-	-	_	528
-	_	ctg Leu	-		_	-	-	_	_		_	-	-	-		576
		aag Lys 195			_			-		_		-	-		-	624
		aag Lys			-			-				-	_		-	672
	-	cgc Arg			-											696
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<211> 232

<212> PRT

<213> Toxoplasma gondii

<400> 116

- Arg Gly Asn Glu Lys Thr Cys Ser Asp Ala Lys His Pro Val Tyr Ile

 1 5 10 15
- Lys Leu Gly Lys Gly Glu Arg Glu Ala Val Phe Lys Cys Gly Asp Gly
 20 25 30
- Leu Thr Thr Leu Glu Pro Ser Gln Asn Thr Asp Lys Pro Lys Phe Cys
 35 40 45
- Glu Ser Ile Asp Cys Asn Asp Thr Ala Glu Leu Glu Thr Thr Phe Pro
 50 55 60
- Gly Ala Tyr Trp Asp Glu Arg Asn Lys Lys Ala Asn Ile Tyr Arg Leu 65 70 75 80
- Val Ile Pro Thr Val Ser Arg Lys Asp Thr Arg Met Tyr Tyr Lys Cys 85 90 95
- Lys Gly Thr Ser Asp Ser Ala Asp Pro Cys Thr Val Leu Ile Asn Val 100 105 110
- Lys Ser Thr Glu Thr Asp Asp Glu Glu Glu Asp Val Gln Glu Cys
 115 120 125
- Thr Val Gly Thr Glu Lys Lys Val Thr Leu Ser Pro Thr Asp Thr Val 130 135 140
- Lys Phe Lys Cys Asn Leu Gly Thr Val Val Gln Pro Ser Phe Ser Thr 145 150 155 160
- Ala Thr Pro Lys Val Phe Asp Asp Ser Asp Gly Ser Cys Ser Ala Gln 165 170 175
- Ala Ser Leu Thr Ser Leu Val Asp Ala Ser Leu Thr Glu Asp Ser Ser 180 185 190
- His Gly Lys Tyr Thr Met Tyr Thr Met Asn Leu Asn Ala Arg Pro Ala 195 200 205
- Glu Thr Lys Asn Leu Cys Leu Gln Cys Ser Ser Gly Lys Gln Asn Cys 210 215 220
- Lys Met Arg Ile His Val Pro Ala 225 230

WO 99/32633 PCT/US98/27137 <210> 117

<211> 173

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(171)

<400> 117

act tgt gcg ggg gac ccc tcg gcc ttt ccg acg aag ctg ccg tcg aca 48 Thr Cys Ala Gly Asp Pro Ser Ala Phe Pro Thr Lys Leu Pro Ser Thr 10

cca ccc gct gct gtg ccg tct gac ggg ttg ctc gct ttg ccc tca gaa 96 Pro Pro Ala Ala Val Pro Ser Asp Gly Leu Leu Ala Leu Pro Ser Glu 20

ctt gag gcg ccg gtg gag gac ggc gac cgc gag gct ttc gtt gga gtc 144 Leu Glu Ala Pro Val Glu Asp Gly Asp Arg Glu Ala Phe Val Gly Val

gac ggc gcg gtc agc ggc tgg gac gag cg 173 Asp Gly Ala Val Ser Gly Trp Asp Glu 50 55

<210> 118

<211> 57

<212> PRT

<213> Toxoplasma gondii

<400> 118

Thr Cys Ala Gly Asp Pro Ser Ala Phe Pro Thr Lys Leu Pro Ser Thr 5 10 15

Pro Pro Ala Ala Val Pro Ser Asp Gly Leu Leu Ala Leu Pro Ser Glu 20 25 30

Leu Glu Ala Pro Val Glu Asp Gly Asp Arg Glu Ala Phe Val Gly Val 40

Asp Gly Ala Val Ser Gly Trp Asp Glu 50 55

<210> 119

<211> 369

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(369)

<400> 119

cgc tct gtg ttt cag gtc gcg agc gac gcg aga aac gcc cga cag gcg 48 Arg Ser Val Phe Gln Val Ala Ser Asp Ala Arg Asn Ala Arg Gln Ala 1 5 10 15

acc tcg ggc gtg ccg cgg cag agg gga aag aag gcc gtc acg gcg cga 96
Thr Ser Gly Val Pro Arg Gln Arg Gly Lys Lys Ala Val Thr Ala Arg
20 25 30

gtc tct ttc ggc gct cta gag gag aga gac agt tcg agt tcg gac gtt 144 Val Ser Phe Gly Ala Leu Glu Glu Arg Asp Ser Ser Ser Ser Asp Val 35 40 45

ccc gag gaa agg gat aaa gac gcc gaa aac ggc tct gcg cct cgc atc 192 Pro Glu Glu Arg Asp Lys Asp Ala Glu Asn Gly Ser Ala Pro Arg Ile 50 55 60

ttc gcg tct tct tcc ctg acg cgg ctt tcg cct cct tct ctc tct ccg

Phe Ala Ser Ser Ser Leu Thr Arg Leu Ser Pro Pro Ser Leu Ser Pro

65 70 75 80

ctc tca agt tcg ggg cca tct tca ccg tct tct tcc gtt tcg cgg ttt 288
Leu Ser Ser Ser Gly Pro Ser Ser Pro Ser Ser Ser Val Ser Arg Phe
85 90 95

acc gac tcc ctg ccg cag tcg acg gct tcg tct cgt ctc tcc tct gct 336 Thr Asp Ser Leu Pro Gln Ser Thr Ala Ser Ser Arg Leu Ser Ser Ala 100 105 110

tat tcg ctt gag tcg cgt cgg cct ctg gag ccg

Tyr Ser Leu Glu Ser Arg Arg Pro Leu Glu Pro

115 120

<210> 120

<211> 123

<212> PRT

<213> Toxoplasma gondii

<400> 120

Arg Ser Val Phe Gln Val Ala Ser Asp Ala Arg Asn Ala Arg Gln Ala

WO 99/32633			PCT/US98/27137
1	5	10	15

Thr Ser Gly Val Pro Arg Gln Arg Gly Lys Lys Ala Val Thr Ala Arg
20 25 30

Val Ser Phe Gly Ala Leu Glu Glu Arg Asp Ser Ser Ser Ser Asp Val 35 40 45

Pro Glu Glu Arg Asp Lys Asp Ala Glu Asn Gly Ser Ala Pro Arg Ile 50 55 60

Phe Ala Ser Ser Ser Leu Thr Arg Leu Ser Pro Pro Ser Leu Ser Pro 65 70 75 80

Leu Ser Ser Ser Gly Pro Ser Ser Pro Ser Ser Ser Val Ser Arg Phe
85 90 95

Thr Asp Ser Leu Pro Gln Ser Thr Ala Ser Ser Arg Leu Ser Ser Ala 100 105 110

Tyr Ser Leu Glu Ser Arg Arg Pro Leu Glu Pro 115 120

<210> 121

<211> 566

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(183)

<400> 121

cgg cgg tgg atg acg ggt gct aat tac gag ggc cac cag gga caa tat 48 Arg Arg Trp Met Thr Gly Ala Asn Tyr Glu Gly His Gln Gly Gln Tyr 1 5 10 15

ttg aac tac tgc acc att tct cac ttc ttg tgt tgc cct aat ggg atc 96 Leu Asn Tyr Cys Thr Ile Ser His Phe Leu Cys Cys Pro Asn Gly Ile 20 25 30

tgt cgt ttt caa tgg gac aat cag ccc agt ctc gat agg gag gac tca 144 Cys Arg Phe Gln Trp Asp Asn Gln Pro Ser Leu Asp Arg Glu Asp Ser 35 40 45

atc tgg tgc tct gaa tcg att tct cgt ttt cgc ctg agc taagataact 193 Ile Trp Cys Ser Glu Ser Ile Ser Arg Phe Arg Leu Ser

50 55 60

getgaagaca tttgtagacg etttetacaa acceaegtgg caaaatetta eggaaggaca 253
aatgeetett teaacaetet tetteeateg etgettgtta eaeteetgag aggeeecaag 313
ageeaeggtg ceaetttget teeceageeg etaetgtga aattetttat agaagageae 373
aaatgtteee egaagaagea geageaeeet ttgaggagee tgaagagega eeetaegaat 433
cacagegtte agaaatagee taetgtagta ttaaggagae taecaaagtg aaaategtga 493
tatgtetaca ggtggtatge aagtgttggt ttteeagata taegetgea etaaaacaee 553
aaaatgatag aat 566

<210> 122

<211> 61

<212> PRT

<213> Toxoplasma gondii

<400> 122

Arg Arg Trp Met Thr Gly Ala Asn Tyr Glu Gly His Gln Gly Gln Tyr

1 5 10 15

Leu Asn Tyr Cys Thr Ile Ser His Phe Leu Cys Cys Pro Asn Gly Ile
20 25 30

Cys Arg Phe Gln Trp Asp Asn Gln Pro Ser Leu Asp Arg Glu Asp Ser 35 40 45

Ile Trp Cys Ser Glu Ser Ile Ser Arg Phe Arg Leu Ser 50 55 60

<210> 123

<211> 616

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(615)

<400> 123

cac gag cgc cgt gtg gca gag caa aag gct cgt gaa gaa cgc gag aga 48 His Glu Arg Arg Val Ala Glu Gln Lys Ala Arg Glu Glu Arg Glu Arg WO 99/32633 PCT/US98/27137

1 5 10 15

1				5					10					15		
		gca Ala														96
		tgt Cys 35														144
		tgc Cys														192
		tca Ser														240
		tcg Ser														288
gac Asp	cgg Arg	att Ile	tct Ser 100	gcg Ala	gag Glu	gag Glu	gcg Ala	gcg Ala 105	tcc Ser	ctc Leu	gag Glu	gag Glu	gcc Ala 110	cgg Arg	cgg Arg	336
		aga Arg 115														384
		tac Tyr														432
		tgg Trp														480
gcg Ala	gcg Ala	tcg Ser	atg Met	aga Arg 165	agt Ser	gac Asp	ggt Gly	cgc Arg	aga Arg 170	ggt Gly	caa Gln	cag Gln	ccc Pro	ccg Pro 175	agt Ser	528
cga Arg	cag Gln	tct Ser	cct Pro 180	cag Gln	gac Asp	Gly	gag Glu	gaa Glu 185	gac Asp	gac Asp	gcc Ala	gct Ala	ctc Leu 190	gcc Ala	aga Arg	576
		cag Gln											g			616

195 200 205

<210> 124

<211> 205

<212> PRT

<213> Toxoplasma gondii

<400> 124

His Glu Arg Arg Val Ala Glu Gln Lys Ala Arg Glu Glu Arg Glu Arg
1 5 10 15

Gln Ala Ala Ser Gln Arg Asn Gly Ser Thr Glu Pro Ala Val Ala Pro 20 25 30

Ser Ser Cys Ser Ser Ser Asn Ser Gln Asn Pro Pro Gln Asp Ser Ser 35 40 45

His Val Cys Cys Pro Ser Ser Ser Ala Phe Ser Gln Pro Arg Ser Ser 50 55 60

Leu Ser Ser Ser Ser Pro Ser Ser Ser Ala Ala Leu Pro Ser Gly Ser 65 70 75 80

Ser Pro Ser Ala Ala Ser Ser Ser His Ala Leu Gly Val Val Asp Ser 85 90 95

Asp Arg Ile Ser Ala Glu Glu Ala Ala Ser Leu Glu Glu Ala Arg Arg
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85 90 95

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Glu Glu Met His Glu Ala Tyr Asp Pro Leu Leu Glu Fhe Val Glu Thr
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Phe Arg Glu Ile Lys Lys Ala Val Glu Glu Asp Ala Ala Leu Ser Thr 65 70 75 80

Asp Ala Ile Asp Arg Val Ser Gln Phe Asp Leu Val Ser Leu Leu Asp 85 90 95

Val Ile Arg Glu Ala Ala Gln Ala Lys Phe Asp Leu Leu Gly Arg Leu 100 105 110

Ile Thr Asp Ile Ala Ser Gly Ile Gly Glu Gly Ala Met Ala Leu Met 115 120 125

Gly Glu Glu Ala Ala Phe Ile Arg Pro Arg Arg Ser Lys Arg Gly Lys 130 135 140

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gaa Glu	- •						c gac r Asp 5									429
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gat Asp			į		: Vā		c cag r Gln									525

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Glu Glu Met His Glu Ala Tyr Asp Pro Leu Leu Glu Phe Val Glu Thr
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Phe Arg Glu Ile Lys Lys Ala Val Glu Glu Asp Ala Ala Leu Ser Thr 65 70 75 80

Asp Ala Ile Asp Arg Val Ser Gln Phe Asp Leu Val Ser Leu Leu Asp 85 90 95

Val Ile Arg Glu Ala Ala Gln Ala Lys Phe Asp Leu Leu Gly Arg Leu
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Ile Thr Asp Ile Ala Ser Gly Ile Gly Glu Gly Ala Met Ala Leu Met 115 120 125

Gly Glu Glu Ala Ala Phe Ile Arg Pro Arg Arg Ser Lys Arg Gly Lys 130 135 140

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ational Application No PCT/US 98/27137

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/30 C07K14/45

A61K48/00 A61K39/002 C12N15/11 A61K39/395

C12N5/10 C12Q1/68

C07K16/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A61K C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUM	ENTS CONSIDERED TO BE RELEVANT	
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 516 381 A (MERCK & CO INC) 2 December 1992 see examples 3,4,6	39-42
Α	EP 0 687 471 A (BAYER CORPORATION) 20 December 1995 see the whole document	1-38
Α	EP 0 710 724 A (AKZO NÖBEL N. V.) 8 May 1996 see page 7 – page 9	1-38
Α	EP 0 700 991 A (BAYER CORPORATION) 13 March 1996 see the whole document/	1-38

Further documents are listed in the continuation of box C.	Patent family members are listed in annex.
"A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family
Date of the actual completion of the international search 19 April 1999	Date of mailing of the international search report $03/05/1999$
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Mandl, B

Form PCT/ISA/210 (second sheet) (July 1992)



Int. Ational Application No PCT/US 98/27137

Citation of document with indication where appropriate of the relevant passages	Relevant to claim No.
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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

international application No.

PCT/US 98/27137

Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet) This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons: | X | Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely: Remark: Although claims 9 and 32-38 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition. because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically: Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a). Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet) This International Searching Authority found multiple inventions in this international application, as follows: As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.: No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: Remark on Protest The additional search fees were accompanied by the applicant's protest. No protest accompanied the payment of additional search fees.





Inte. Jonal Application No PCT/US 98/27137

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(54) Title: TOXOPLASMA GONDII PROTEINS, CORRESPONDING NUCLEIC ACID MOLECULES, AND USES THEREOF

(57) Abstract

The present invention relates to immunogenic Toxoplasma gondii proteins, to T. gondii nucleic acid molecules, including those that encode such proteins and to antibodies raised against such proteins. The present invention also includes methods to obtain such proteins, nucleic acid molecules and antibodies. Also included in the present invention are compositions comprising such proteins, nucleic acid molecules and/or antibodies, as well as the use of such compositions to inhibit oocyst shedding by cats due to infection with T. gondii. The present invention also includes the use of certain T. gondii-based antisera to identify such nucleic acid molecules and proteins, as well as nucleic acid molecules and proteins identified by such methods. The present invention also relates to methods for the detection of cysts and oocvsts.

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